AWARD NUMBER: W81XWH-11-1-0374

TITLE: "Opioid Abuse after Traumatic Brain Injury: Evaluation Using Rodent Models."

PRINCIPAL INVESTIGATOR: Katherine L. Nicholson

CONTRACTING ORGANIZATION: Virginia Commonwealth University
Richmond, VA 23298

REPORT DATE: September 2015

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED
September 2015	Final	1Jul2011 - 30Jun2015

4. TITLE AND SUBTITLE

"Opioid Abuse after Traumatic Brain Injury: Evaluation Using Rodent Models."

5a. CONTRACT NUMBER
W81XWH-11-1-0374

5b. GRANT NUMBER
PR100379P1

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Katherine L. Nicholson

email: klnichol@vcu.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

Virginia Commonwealth University
Office of Sponsored Programs 800
East Leigh Street, Suite 113 PO Box
980568 Richmond, VA 23298-0568

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

This project evaluated the effect of a moderate-level brain injury on risk for opioid abuse using preclinical models in rats. We assessed the effect of brain injury on the rewarding effects of oxycodone in three rat self-administration procedures and found significant differences in the acquisition and maintenance of oxycodone self-administration behavior between brain-injured and control rats. Data showed brain injured rats acquired self-administration behavior at lower doses, took higher total doses of oxycodone and worked significantly harder for a single infusion of drug suggesting oxycodone had stronger rewarding effects following injury. Conversely, brain-injured subjects showed lower responding in a model of relapse to oxycodone self-administration. Testing of oxycodone for analgesic strength and development of tolerance showed no difference between sham controls and brain injured subjects. Nor was there any difference detected in the development of dependence. Overall, the analgesia studies demonstrate that moderate brain injury does not result in an altered pain state or diminished response to oxycodone analgesia and the dependence studies show withdrawal is not more severe. However, the self-administration studies suggest that brain-injured subjects could be at increased risk for developing opioid substance abuse disorders.

15. SUBJECT TERMS
Traumatic brain injury, drug abuse, oxycodone, opioid, preclinical models, rat

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	58	19b. TELEPHONE NUMBER *(include area code)*

Table of Contents

Page

Final Report for DoD Peer Reviewed Medical Research Program of the Office of the Congressionally Directed Medical Research Program FY10 Investigator-Initiated Research Award: Partnering PI Option Application entitled "Opioid Abuse after TBI"

Prepared by Katherine L. Nicholson, DVM, Ph.D.

Introduction:

Data from both military and civilian studies indicate that drug abuse rates are increased by the occurrence of a traumatic brain injury (TBI), but the underlying reasons for this remain unclear. This relationship between TBI and drug abuse is particularly alarming given the significant numbers of military personnel who experience a brain injury and the growing numbers of persons who are prescribed opioid pain medications. There is significant overlap in anatomical brain regions involved in reward pathways associated with addiction and the brain regions commonly damaged in TBI which suggests that TBI could alter the reward circuitry, thereby increasing the likelihood of opioid abuse and addiction. Given the overlap in affected brain regions and growth factors, we speculated that TBI could result in neurological changes that increase vulnerability for drug abuse and addiction. Consequently, we have been evaluating the effects of TBI on both the rewarding effects of opioid drugs as well as the development of tolerance and physical dependence in well-established rat models of abuse-related drug effects. We used lateral fluid percussion injury to induce a moderate level of injury in adult, male Sprague-Dawley rats. Our opioid compound selected for study is one of the most commonly prescribed and also misused/abused prescription pain medications, oxycodone. To assess the rewarding effects of oxycodone after TBI, we used intravenous self-administration procedures to evaluate 1) the acquisition and maintenance of oxycodone self-administration as well as 2) the risk of relapse to oxycodone self-administration following extinction of the behavior and 3) the reinforcing strength of oxycodone as measured by self-administration behavior under a progressively increasing work requirement. We have found that there are significant differences in the acquisition and maintenance of self-administration behavior between brain-injured and control rats. Data collected to date suggest brain injured rats have a greater sensitivity to the rewarding effects of oxycodone and will self-administer greater total doses of drug compared to sham controls. Additionally, when the work requirement for each infusion of drug is increased, brain injured subjects elicited far more responses suggesting oxycodone has stronger rewarding effects following brain injury. In a model of relapse to oxycodone abuse, while the brain-injured rats required significantly longer to discontinue responding when oxycodone was no longer available (stronger drug seeking behavior), once responding was decreased it was not as easily reinstated in the brain-injured subjects. This suggests a possible reduced propensity for relapse in patients who have experienced a TBI. We have also investigating the acute analgesic effects of oxycodone after TBI and the effect of repeated oxycodone administration. Testing for analgesic strength and development of tolerance has shown no difference between sham controls and brain injured subjects using either a spinally mediated or a supraspinally mediated pain model. Similarly, studies to determine the effect TBI has on the development of physical

dependence did not support significant differences between brain injured and sham injured controls. Overall, while the results in the analgesia / tolerance studies and the dependence studies are encouraging regarding the treatment of pain in TBI patients, the results in the self-administration studies suggest that brain-injured subjects are at higher risk for developing opioid abuse disorders.

Body:

The study has been finalized and the data generated in completion of the Aims is described below. The original Aim with a brief description (underlined font), the related SOW Task (italicized font) and the work conducted (normal font) towards that Aim and Task during year 4 are also summarized below. The animal numbers described are based on a loss of 5% of subjects following sham brain injury and 20% loss following moderate injury.

Aim 1: Evaluate the hypothesis that moderate TBI causes changes in analgesic response to opioids following acute and repeated drug administration. Separate groups of adult male rats will undergo either sham (control) injury or lateral fluid percussion injury of moderate severity. We will use hot plate-induced paw withdrawal (HP) and warm water tail withdrawal (WWTW) models to measure the antinociceptive activity of oxycodone after TBI/sham injury. To evaluate tolerance development, oxycodone will be chronically administered via microinfusion pump after TBI. Oxycodone antinociception will be re-evaluated after chronic oxycodone exposure and the dose effect curves compared. Significant decrease in potency following chronic exposure will be indicative of tolerance development.

- *SOW Task 1 General: For the tolerance and dependence procedures, the goal was to generate 10 subjects per treatment condition as outlined in the research design for a total of 40 test subjects completing evaluation of the antinociceptive effects of oxycodone following acute and chronic administration and 40 subjects completing assessment of development of physical dependence. The 10 subjects completing each treatment condition were euthanized following the final oxycodone exposure for collection of brains for analysis.*

- *SOW Task 1 Year 4 Specific: 8 additional rats completed testing of the antinociceptive effects of oxycodone and the development of tolerance using the WWTW (4 rats) and hotplate test (4 rats) providing 10-11 subjects/condition in each pain model. These subjects were euthanized and brains collected for shipment to Dr. Floyd at UAB following the final oxycodone testing.*

- Work Completed: An additional 10 subjects were implanted with iPrecio computerized mini pumps in order to ensure completion in minimally six subjects during this final round of testing. Of these, 8 (4 WWTW, 4 HP) completed testing in the analgesia and

tolerance procedures and had brain tissue collected. Details of the group numbers as well as a review of the data are listed in Sections 3 and 4.

- Completed testing in WWTW: 5 entered the protocol, 4 finished (goal: 5 enter, 3 finish). All testing has been completed.
- Completed testing in hotplate procedure: 5 entered the protocol, 4 finished (goal: 5 enter, 3 finish). All testing has been completed.
-

Aim 2: Investigate the hypothesis that moderate TBI increases the susceptibility for opioid abuse as measured by an alteration in the rewarding properties of oxycodone. Using 3 intravenous self-administration (SA) procedures we will evaluate the reinforcing effects of oxycodone following induction of TBI. The rate of acquisition of oxycodone S-A, the reinforcing efficacy of oxycodone, and the vulnerability to relapse to self-administration of oxycodone will be determined following sham or moderate brain injury in separate groups of rats.

- *SOW Task 2 General: Each cohort of self-administration (SA) animals will require 35 to 60 days to complete testing depending on the aspect of SA being assessed. This includes time for acclimation to the laboratory and handling, catheterization surgery and recovery, brain injury and evaluation of acquisition, reinforcing efficacy or reinstatement to oxycodone SA. This time frame is based on exclusive use of 4 self-administration chambers in 2 or 3 runs/day = 8 to 12 animals/day. Therefore, 8 to 12 rats can undergo injury and be designated to a SA procedure every 6 to 8 weeks. For all SA studies, the numbers shown below reflect the number of animals entering the different SA paradigms. With an anticipated loss of ~25% of subjects due to premature loss of catheter patency (acquisition) and failure to acquire the baseline behavior (PR and reinstatement procedures) this will result in a total of 10 subjects/treatment group.*

- Work Completed: Testing of subjects in the three self-administration procedures, acquisition, reinstatement and reinforcing efficacy, was complete by the end of Y3. All testing in food maintained subjects was also completed in Y3.

Aim 3: Evaluate the propensity for development of physical dependence to opioids following moderate TBI. We will evaluate development of dependence following chronic administration of oxycodone in both sham and TBI rats for differences in the dose and duration of exposure required to produce dependence as well as severity of withdrawal. Dependence will be measured by scoring of withdrawal signs as well as assessing the disruption of operant responding for food reward following both spontaneous and precipitated withdrawal from oxycodone.

- *SOW Task 3 General: For the tolerance and dependence procedures, the goal is to generate 10 subjects per treatment condition as outlined in the research design for a total of 40 test subjects completing evaluation of the antinociceptive effects of*

oxycodone following acute and chronic administration and 40 subjects completing assessment of development of physical dependence. The 10 subjects completing each treatment condition will be euthanized following the final oxycodone exposure for collection of brains for analysis.

- Work completed: The physical dependence study was deferred to year three in the SOW modification in order to redistribute costs to accommodate added subjects in the self-administration study. Work on this task began in February 2014 and was completed during the period of the requested and approved no cost extension. Details of the group numbers as well as a review of the data are listed in Section 9.
    - Completed testing in dependence procedure: 40 entered the protocol, 38 reached criteria for acquisition of operant procedure before they were too large to undergo craniectomy/injury, 34 survived the injury procedure and completed behavioral testing (goal: 40 enter, 31 finish). All testing has been completed.

- ***1. Overview of milestones completed in FY4***

Table 1. Shown is the distribution of animals entered into the study during project Y4 (no cost extension) from 07/01/14 through 07/31//15. Numbers in the final column in parentheses denote the total number completed across the three years / total number to be completed by project end.

<table>
<tr><td rowspan="15">Total # subjects entered into protocol

=50</td><td rowspan="6">Total number catheterized

= 0</td><td rowspan="3">Total number undergoing sham injury

=0</td><td>Total entering acquisition
= 0</td><td>Total completing acquisition
= 0 (40/40)</td></tr>
<tr><td>Total entering reinstatement
= 0</td><td>Total completing reinstatement
= 0 (10/10)</td></tr>
<tr><td>Total entering PR evaluation
=0</td><td>Total completing PR evaluation
= 0 (10/10)</td></tr>
<tr><td rowspan="3">Total number undergoing TBI
= 0</td><td>Total entering acquisition
= 0</td><td>Total completing acquisition
= 0 (40/40)</td></tr>
<tr><td>Total entering reinstatement
= 0</td><td>Total completing reinstatement
= 0 (12/10)</td></tr>
<tr><td>Total entering PR evaluation
=0</td><td>Total completing PR evaluation
=0 (10/10)</td></tr>
<tr><td rowspan="4">Total number implanted with infusion pumps
= 10</td><td rowspan="2">Total number undergoing sham injury
= 0</td><td>Total number entering WWTW testing = 0</td><td>Total completing testing/dosing
= 0 (20/20)</td></tr>
<tr><td>Total number entering HP testing = 0</td><td>Total completing testing/dosing
= 0 (20/20)</td></tr>
<tr><td rowspan="2">Total number undergoing TBI
= 10</td><td>Total number entering WWTW testing =4</td><td>Total completing testing/dosing
= 4 (21/20)</td></tr>
<tr><td>Total number entering HP testing = 4</td><td>Total completing testing/dosing
= 4 (21/20)</td></tr>
<tr><td rowspan="2">Total number allocated to Food-maintained behavior and locomotor activity = 0</td><td>Total number undergoing sham injury =0</td><td>Total number entering testing
= 0</td><td>Total completing testing
= 0 (10/10)</td></tr>
<tr><td>Total number undergoing TBI
= 0</td><td>Total number entering testing
= 0</td><td>Total completing testing
= 0 (10/10)</td></tr>
<tr><td rowspan="2">Total number completing training for the dependence operant procedure
= 38</td><td>Total number undergoing sham injury = 17</td><td>Total number entering dependence testing = 17</td><td>Total number completing testing and dosing
= 17 (21/20)</td></tr>
<tr><td>Total number undergoing TBI
= 21</td><td>Total number entering dependence testing = 17</td><td>Total number completing testing and dosing
= 17 (22/20)</td></tr>
</table>

2. Subjects:

Adult male Sprague Dawley rats were purchased from Charles River at age/size ranges predicted to result in body weights of 290-330 g at time of injury. Self-administration, food acquisition/locomotor activity and hot plate antinociception subjects were purchased minimally 2 weeks prior to injury permitting time for 7 days of handling and acclimation before intravenous catheterization or pump implantation was performed (see below). Subjects to be used in the warm water tail withdrawal procedure (WWTW) were delivered minimally 3 weeks prior to scheduled injuries in order to fully acclimate them to the restraint tubes. Subjects for the dependence procedure required extensive pre-injury training and were purchased 1 month prior to undergoing craniectomy.

3. Fluid percussion Injury:

All subjects underwent the injury procedure following handling, training and surgical instrumentation for subsequent behavioral procedures. Anesthesia was induced and maintained with 4% isoflurane. The subjects were surgically prepared and transferred to a stereotaxic device for craneictomy and continued to be maintained under isoflurane anesthesia.
Lateral fluid percussion injury was then induced in approximately 50% of the rats as previously described (Floyd et al., 2002).

Description of Procedure. An incision (~8mm) was made in scalp and fascia scraped from the skull. A point mid-way between Bregma and Lambda and central suture/lateral ridge was marked on the medial skull surface with sterile tissue marker. A 4.8mm craniotomy was cut with a trephine by hand over the right motor cortex. An injury cannula was fashioned from the hub of a female leur-lock 20g needle by affixing the plastic tube to the skull with glue and securing with dental acrylic. After the acrylic hardened (15 minutes), the injury cannula was filled with sterile saline, and the brain injury induced by compressing the sterile saline with the fluid percussion device (Custom Design and Fabrication, VCU, Richmond, VA) controlled to deliver an equivalent impact to each animal of moderate (2.5ATM) severity. After induction of TBI, the scalp was sutured w/ 4-0 PDS and the animal was returned to a clean, warmed, home cage when ambulatory. Sham control animals underwent all procedures with the omission of the fluid percussion pulse.

Post-TBI analysis of transient loss of consciousness. Analysis of righting reflex suppression is an indicator of duration of loss of consciousness after TBI. Counting of time until return of consciousness began immediately after the percussion injury. When a conscious rat is placed on its back, it will flip to its feet or "right" itself (Floyd et al., 2002). Time to return of righting reflex after TBI was recorded and used as an indicator of loss of consciousness, a valid measure of injury severity. As shown below (Table 2, Figure 1), **the loss of consciousness for the subjects undergoing a lateral fluid percussion injury of moderate severity was greater than 2-fold longer than that for sham injured subjects**, consistent with a moderate level of

brain injury. Comparison using the student's t test verified a significant difference between injury groups ($p < 0.001$). Subjects had to have righting times within 2 standard deviations (St Dev) of the injury group mean in order to qualify for inclusion in the final data analysis.

Table 2. Righting times across injury groups with St Dev and SEM calculations.

	Moderate Injury	Sham
Mean Righting time (sec)	667.8	270.3
St Dev	147.8	63.3
SEM	13.4	6.5

Following injury, subjects were monitored closely and recovery performance recorded for 5 days. All subjects received 3 ml saline containing 5 mg/kg enrofloxacin SC, daily for 3 days. After 5 days, subjects began evaluation for the behavioral effects of oxycodone towards completion of Aims 1, 2 and 3 as described below.

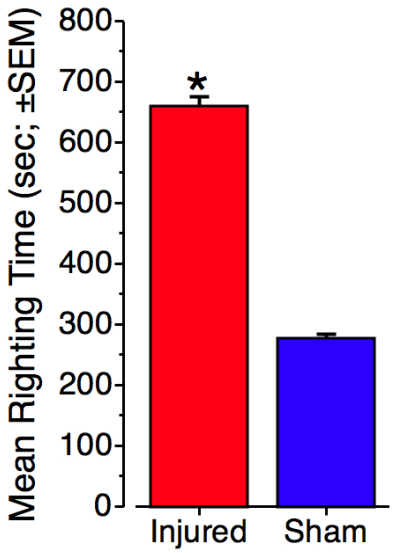


Figure 1. Shown are the mean righting times (sec; ±SEM) for all subjects undergoing either a lateral fluid percussion injury of moderate severity (Injured) or a sham injury (Sham).

4. Tasks performed specific to Aim 1 – evaluation of acute antinociceptive effects of oxycodone and development of tolerance using a WWTW procedure

One series of experiments towards achieving this Aim involved use of a warm water tail withdrawal (WWTW) procedure. Prior to injury and testing, the subjects were habituated to placement in a specially designed rodent restraint tube (Braintree Scientific, Braintree, MA) with their tails hanging freely out the caudal end. All subjects underwent 2 to 3 weeks of acclimation to the tubes beginning with 10 min of restraint/day and increasing to 60 min of restraint/day. Once at 60 min/day the animals continued to be habituated to the tubes every other day for 60 min until the time of injury. Habituation was suspended for 3 days following craniectomy/injury to avoid any possible effect on post injury recovery. On day 4 post-TBI,

subjects were habituated for 60 min and on day 5 post-TBI, antinociception testing with oxycodone began. Thus all subjects were well acclimated to the tubes prior to antinociception testing and displayed no evidence of stress associated with the restraint. In fact subjects readily entered the tubes and were difficult to extract at the end of each habituation period.

On test days, the distal 5 cm of the tail was immersed in containers of water of different temperatures. To qualify for testing the rat had to leave his tail immersed in 40^{o} C water for 12 sec during 2 of 3 repeated exposures with 2 min between exposures. This is a non noxious stimulus and once the animals have been habituated to the restraint and to the sensation of tail immersion, almost all subjects qualify. During oxycodone testing, the subjects were exposed to noxious water temperatures of 50^{o} and 55^{o}C, consistent with our previous work (Morgan and Nicholson, 2011) and the latency to withdraw the tail was recorded. Through the use of cumulative dosing, an entire oxycodone dose effect curve was determined over approximately 2 hours. Based on procedural issues that were noted during Y1, several minor changes were made to improve reliability of data generation while also permitting retention of previously generated data. During WWTW testing in Y1 we experienced problems with tail irritation/inflammation following the initial oxycodone dose-effect curve determination. This resulted in an allodynic response for the 40^{o}C qualifying test. This painful response to a non noxious stimulus resulted in exclusion of a number of subjects from subsequent testing, particularly for the day 11 post-TBI oxycodone testing. To minimize this problem we decreased the cutoff time for tail withdrawal from 15 to 12 sec. Additionally, following testing all subjects were placed in restraint tubes daily and tails treated with topical antibiotic/steroid ointment to minimize any inflammatory response. With these small changes in place, no subjects missed test days or were removed from the study due to tail lesions during the remainser of the study. Data generated during Y1 was reanalyzed using the 12 sec cutoff time in order to retain the data generated from as many subjects as possible. However, seven subjects (5 sham controls and 2 brain-injured) that were not able to complete a dose-effect curve beyond the initial curve during Y1 were excluded from analysis and replaced in Y2. The data and numbers presented below do not include the subjects which completed testing under ED_{50} conditions or the subjects that were removed from data analysis retrospectively.

To summarize, the subjects were slated to undergo the following in order:

1. Habituation and training in the procedure.
2. Implantation of iPrecio pre-programmed mini pumps.
3. Sham or lateral fluid percussion injury.
4. Determination of an oxycodone dose-effect curve.
5. Repeated daily dosing every 6 hours with oxycodone or saline via the iPrecio mini pump.
6. Redetermination of the oxycodone dose-effect curve after 5 days of repeated dosing (day 11 post-TBI).
7. Continue repeated dosing for an additional 5 days.

8. Determine a final oxycodone dose effect curve (day 17 post-TBI).
9. Collect brain tissue for histology and biochemistry at UAB (day 19 post-TBI).

Table 3. Shown are the numbers of subjects assigned to different injury and repeated dosing conditions across Y4 with totals for the study, Y1 – Y4, combined in parentheses. These numbers do not include those removed from final analysis due to incomplete data sets or disqualifying righting times.

<table>
<tr><td rowspan="6">Total number implanted with infusion pumps
= 6 (53)</td><td rowspan="2">Total number undergoing sham injury</td><td colspan="2">Repeated Dosing Assignments</td><td colspan="2">Total completing testing and dosing</td></tr>
<tr><td>Sal</td><td>Oxy
ED80</td><td>Sal</td><td>Oxy
ED80</td></tr>
<tr><td>= 1 (23)</td><td>0 (10)</td><td>1 (11)</td><td>0 (10)</td><td>1 (11)</td></tr>
<tr><td rowspan="2">Total number undergoing TBI</td><td colspan="2">Chronic Dosing Assignments</td><td colspan="2">Total completing testing</td></tr>
<tr><td>Sal</td><td>Oxy
ED80</td><td>Sal</td><td>Oxy
ED80</td></tr>
<tr><td>=5 (30)</td><td>0 (10)</td><td>4 (11)</td><td>0 (10)</td><td>4 (11)</td></tr>
</table>

Pump programming and implantation. Three days prior to induction of TBI, a programmable microinfusion pump (iPrecio system, Data Sciences International, St. Paul, MN) was implanted subcutaneously under isoflurane anesthesia. The rats were placed in ventral recumbency and a 2 cm surgical incision was made longitudinally through the skin along the dorsal midline just caudal to the scapulae. A pocket for the infusion pump was made using blunt dissection directed caudally. The pump was inserted in the pocket and the subcutaneous catheter tubing extending from the pump reservoir attached to a small trocar. The trocar was routed caudad and exteriorized through a small (2mm) skin incision in the lumbar region. This distal end of the tubing was disconnected from the trocar and allowed to retract under the skin. The pump itself was secured to the surrounding fascia and musculature with two 4-0 PDS stay sutures and an additional stay suture was placed around a section of the catheter tubing. The skin incision was closed with wound clips. The pumps were programmed to run with a continuous flush of saline at a rate of 0.2 µl/hour from the time of implantation until repeated dosing began on day 5 post-TBI. Following determination of the oxycodone dose effect curve on that day, the pump reservoir was filled with saline (control group) or an oxycodone solution which provided the ED_{80} dose for the 55^{o} C water stimulus determined during the initial WWTW evaluation in a 30 µl volume. Every 6 hours (approximately 0600,1200,1800 and 0000 hours), 30 µl of saline or oxycodone solution was released in order to mimic clinical exposure. During the intervening hours, the pump continued a low level (0.2µl/hour) flush to maintain patency of the pump tubing. The one exception to this schedule was on days 11 and 17 post-TBI when the 1200 dose was deleted from the program in order not to confound determination of the second and third dose-effect curves. Dosing stopped after the 1200 dose on day 19 post-TBI to permit collection of brain tissue between 2 and 4 hours following dosing, thus avoiding any possible spontaneous

withdrawal effects in the event physical dependence had developed. Pump reservoirs were readily palpated and refilled as needed across the 19-day period.

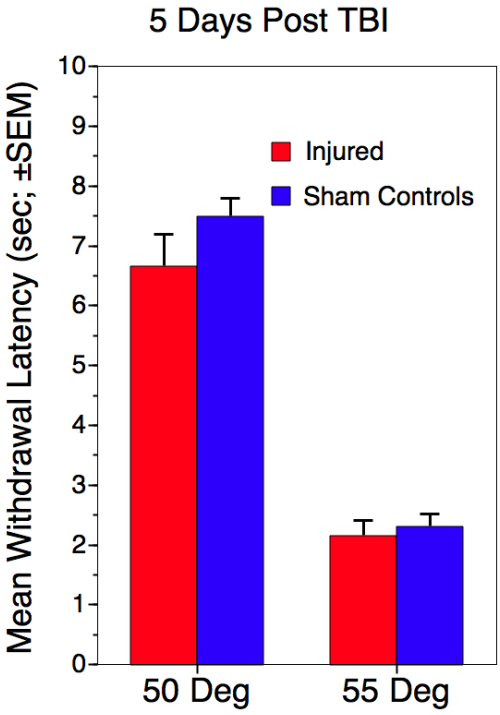


Figure 2. Shown are the baseline tail withdrawal latencies for injured and sham control subjects from 50° and 55° C water. The latencies were measured 15 min after saline administration and served as control values for determination of the % maximum possible effect for all test points following oxycodone administration. The data were collected during determination of the initial oxycodone dose-effect curve on day 5 post-TBI.

Results. Figure 2 presents the baseline tail withdrawal latencies for all brain-injured (n= 21) and sham control (n=21) subjects included in the analyses at the two water temperatures regardless of subsequent dosing group assignment. As predicted, the latencies at 55° were significantly shorter than the corresponding latencies at 50° reflecting the greater intensity of the noxious stimulus. Baseline latencies were used to calculate %MPE = [(test latency - control latency)/ (cut-off time - control latency)] X 100% for each test point. **There was no significant difference in the initial response to the noxious water stimuli between subjects based on injury condition. Thus at day 5 post-TBI there was no indication of either a generalized allodynic or hyperalgesic state in subjects that had received a traumatic brain injury.**

Figures 3 and 4 present the baseline tail withdrawal latencies obtained at the onset of determination of the three oxycodone dose-effect curves (days 5, 11 and 17 post TBI) for brain-injured and sham control subjects that received repeated saline dosing (Figure 3) or the ED_{80} dose of 2.0 mg/kg oxycodone (Figure 4) every 6 hours. Baseline latencies were all within normal ranges across all time points and there were no significant differences between latencies within each injury condition across the three test days. Some trends towards decreasing baseline latencies were noted in some groups, particularly at the lower stimulus intensity, but with one exception these decreases were not significant. The sham controls that received repeated saline displayed a significant decrease in baseline latencies between tests 1 and 2. However, this appeared to be associated with an unusually high latency during test 1 rather than a greater decrease in test 2 relative to the other injury/dosing groups. There was some concern that the

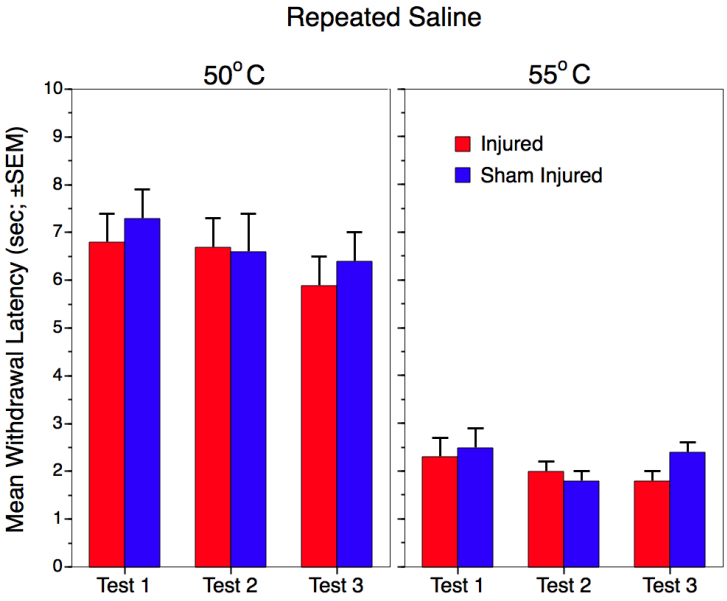


Figure 3. Shown are the baseline tail withdrawal latencies for injured and sham control subjects from 50° and 55° C water. The latencies were measured 15 min after saline administration and served as control values for determination of the % maximum possible effect for all test points. The data were collected at the onset of determination of an oxycodone dose effect curve on days 5 (Test 1), 11 (Test 2) and 17 (Test 3) post-TBI. Test 1 was performed prior to repeated dosing and Tests 2 and 3 were performed after exposure to 5 and 10 days of repeated saline every 6 hours,

trend for decreasing latencies over time was due to a reoccurrence of Y1 problems, however all tails appeared very normal and there was no nociceptive response to the 40°C water nor to moderate pressure applied to the tail tip. Testing was performed 4 hours after the preceding dose and completed within 90 min. This window was gauged to occur when any effects of the previous dose administered had dissipated but withdrawal effects would not have begun. Alternatively these decreased baseline latencies may have been anxiety related. Basically, the subjects appeared to display anticipatory agitation due to their previous experience with the procedure. They were habituated to restraint but not the entire testing process which included SC injections and tail immersions.

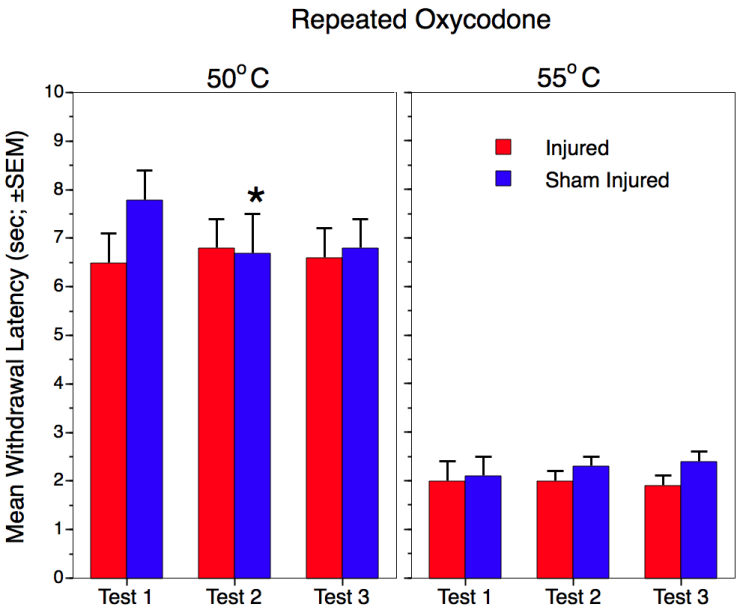


Figure 4. Shown are the baseline tail withdrawal latencies for injured and sham control subjects from 50° and 55° C water. The latencies were measured 15 min after saline administration and served as control values for determination of the % maximum possible effect for all test points. The data were collected at the onset of determination of an oxycodone dose effect curve on days 5 (Test 1), 11 (Test 2) and 17 (Test 3) post-TBI. Test 1 was performed prior to repeated dosing and Tests 2 and 3 were performed after exposure to 5 and 10 days of repeated 2.0 mg/kg oxycodone every 6 hours, respectively. * significantly different from Test 1 at $p < 0.05$.

Figures 5 and 6 present the oxycodone dose-effect curves generated for rats before (post-TBI day 5) and after (post-TBI days 11 and 17) chronic dosing with either saline, or 2.0 mg/kg (Figure 7) oxycodone. As can be seen, **for all treatment/injury conditions oxycodone produced a dose-dependent antinociceptive effect at both stimulus intensities.** What did vary was the relative potency in producing these effects (Tables 4 and 5). As expected, across all treatment/injury conditions, the potency of oxycodone in producing these effects was greater at the lower intensity stimulus. As expected, **following repeated dosing with oxycodone, the dose-effect curves were shifted to the right indicating that oxycodone was less potent in producing its antinociceptive effects and suggesting tolerance had developed.** At the lower stimulus intensity there was greater variability in oxycodone-induced antonociception regardless of injury condition or repeated treatment. Despite the variability, sham control rats that received repeated oxycodone displayed significant curve shifts after both 6 and 12 days of repeated dosing. Similarly the brain-injured rats displayed a significant curve shift after 12 days of repeated dosing. At the higher stimulus intensity repeated 2.0 mg/kg oxycodone resulted in a 2- to 3- fold significant decrease in oxycodone potency regardless of injury condition following both 6 and 12 days of dosing. Unexpectedly the dose effect curves following saline dosing were also shifted to the right to varying degrees. The differences in potency changes were more erratic at the 50^{o}C stimulus and were not consistent across injury conditions. **Overall, while the saline treated animals showed a < 2-fold decrease in oxycodone potency over repeated testing, the oxycodone treated animals showed a 2-fold or greater decrease in potency.** There are several possible causes for the shifts seen in the saline treated animals. An initial concern was that we were continuing to experience imperceptible levels of damage to the tails and hyperalgesia. However, as stated earlier there was no supporting evidence for this. Another possibility is that neuroadaptation associated with tolerance development occurred even after the limited exposure to oxycodone. Acute tolerance has been reported in humans and nonhuman subjects after a single dose of an opioid and is often referred to as acute opioid-induced hyperalgesia (Chu et al., 2008, Lee et al., 2011). A third possible explanation relates to experience with the procedure. Subjectively, the rats appeared more agitated and overtly responsive to injections during tests two and three suggesting that as familiarity with the procedure increased, anticipatory anxiety also resulting in a general hyper-responsiveness to stimuli. Most pertinent to the goals of this project however, when comparing across injury condition, the oxycodone dose effect curves were remarkably similar as shown in Figures 7 and 8. This similarity was present regardless of whether the animals were repeatedly dosed with saline or oxycodone. **Overall, based on the data there does not appear to be a difference in the acute antinociceptive effects of oxycodone or in the development of tolerance to those effects between sham and brain-injured subjects in a model of acute spinally-mediated nociception.**

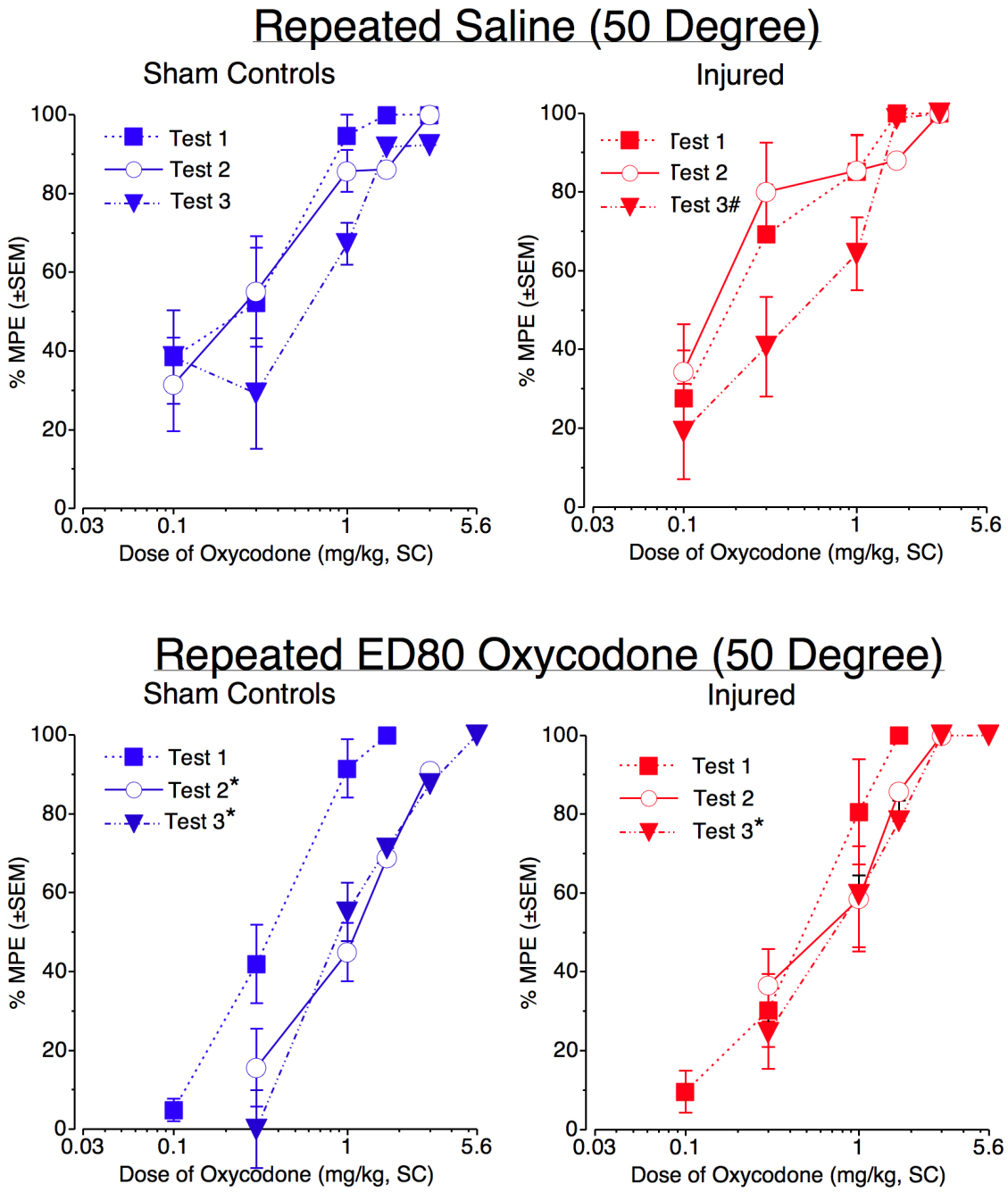


Figure 5. Shown is the percent maximum possible effect for oxycodone antinociceptive effects at the lower stimulus intensity following repeated every 6 hour dosing with saline (top panel) or 2.0 mg/kg oxycodone (bottom panel).

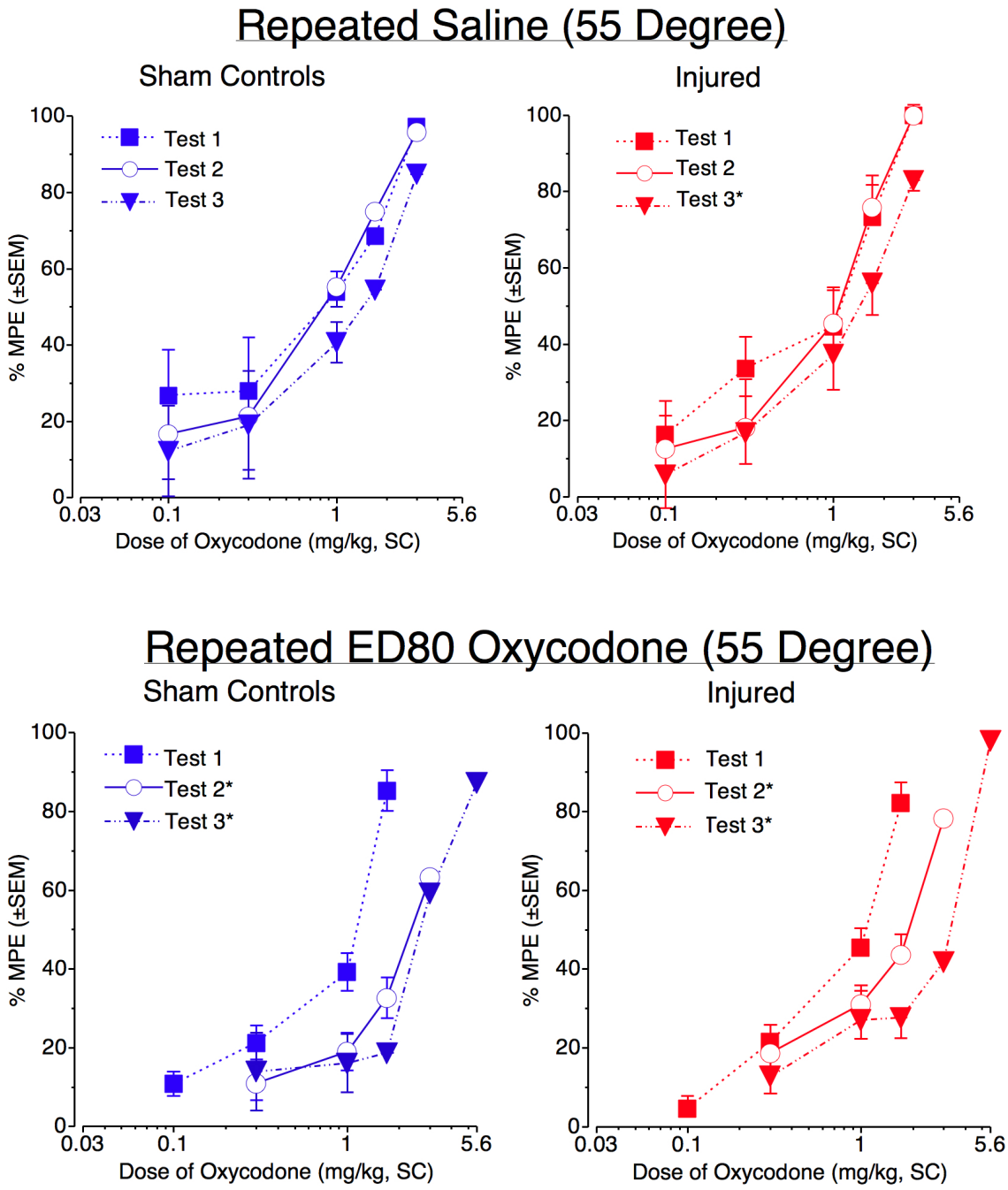


Figure 6. Shown is the percent maximum possible effect for oxycodone antinociceptive effects at the higher stimulus intensity following repeated dosing every 6 hours with saline (top panel) or 2.0 mg/kg oxycodone (bottom panel).

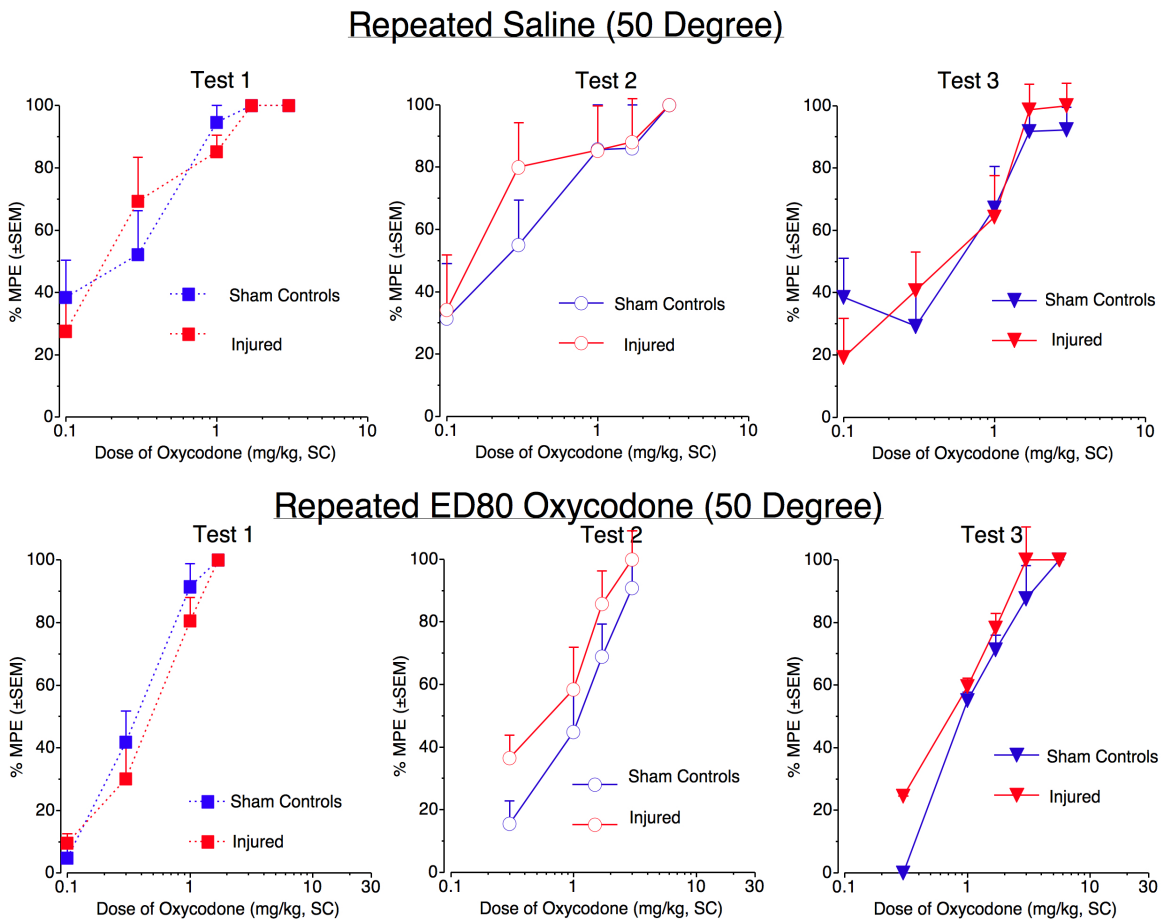


Figure 7. Shown is the percent maximum possible effect for oxycodone antinociceptive effects at the lower stimulus intensity across injury conditions following repeated dosing every 6 hours with saline (top panel) or 2.0 mg/kg oxycodone (bottom panel).

Table 4. Shown are the ED_{50} values (mg/kg) for oxycodone's WWTW antinociceptive effects across both injury conditions prior to and after repeated dosing with saline every 6 hours.

	Test 1		Test 2 after Repeated Saline		Test 3 after Repeated Saline	
Temp (°C)	50°	55°	50°	55°	50°	55°
Injured Subjects	0.20	0.70	0.16	0.86	0.45#	1.19*
95% CL	0.12-0.35	0.50-0.97	0.10-0.26	0.54-1.57	0.33-0.62	0.72-1.97
Sham Controls	0.19	0.74	0.23	0.77	0.33	1.20
95% CL	0.12-0.32	0.56-0.98	0.11-0.47	0.61-0.98	0.18-0.59	0.71-2.03

*significantly different from Test 1 at $p < 0.05$. # significantly different from Test 2 at $p < 0.05$

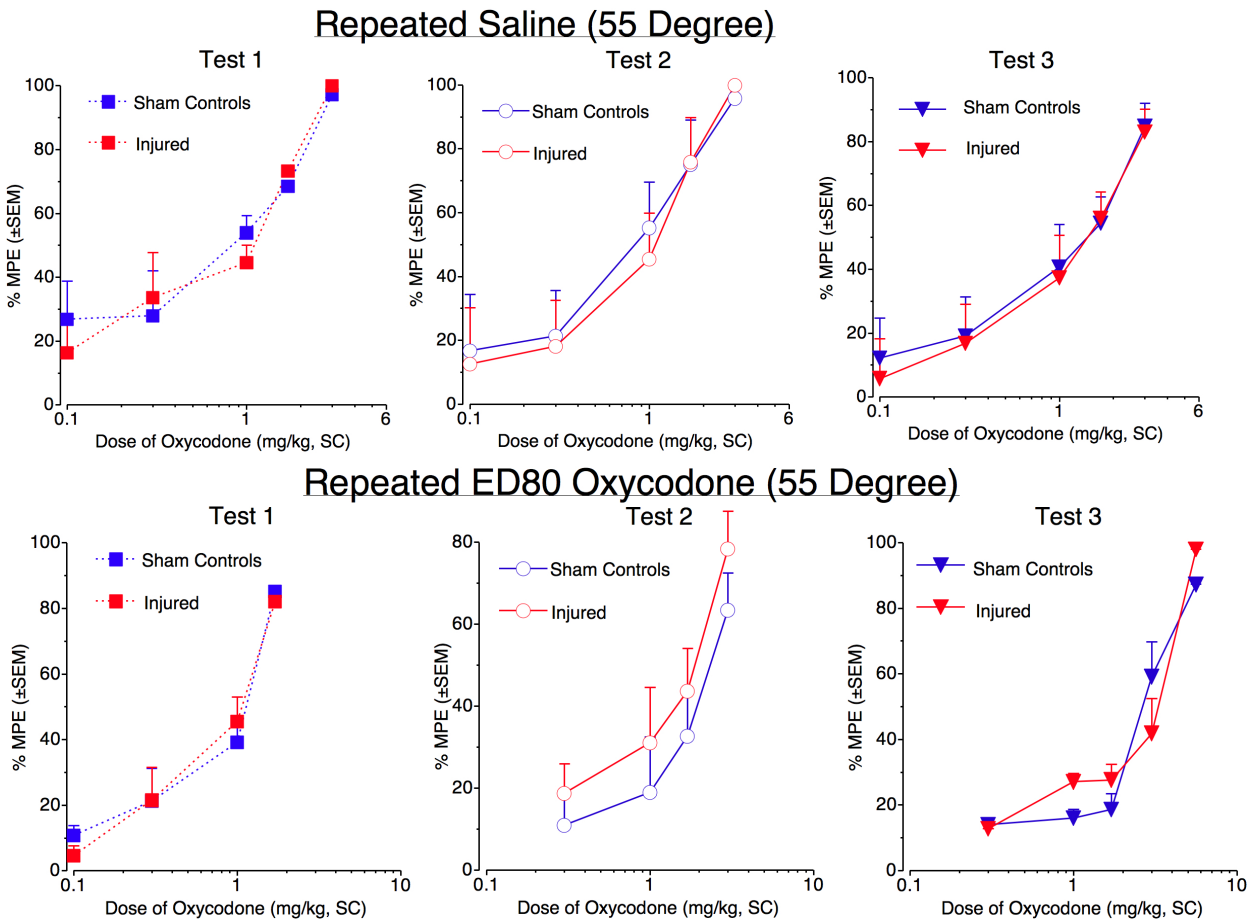


Figure 8. Shown is the percent maximum possible effect for oxycodone antinociceptive effects at the higher stimulus intensity across injury conditions following repeated dosing every 6 hours with saline (top panel) or 2.0 mg/kg oxycodone (bottom panel).

Table 5. Shown are the ED_{50} values (mg/kg) for oxycodone's WWTW antinociceptive effects across both injury conditions prior to and after repeated dosing with the ED_{80} dose of 2.0 mg/kg oxycodone every 6 hours.

	Test 1		Test 2 after Repeated Oxycodone		Test 3 after Repeated Oxycodone	
Temp (°C)	50°	55°	50°	55°	50°	55°
Injured Subjects	0.41	0.80	0.75	1.68*	0.86*	2.21*
95% CL	0.31-0.53	0.61-1.06	0.49-1.14	1.24-2.27	0.65-1.12	1.63-2.98
Sham Controls	0.28	0.83	0.95*	2.29*	0.84*	2.31*
95% CL	0.20-0.39	0.66 - 1.04	0.68 - 1.32	1.81– 2.91	0.51-1.40	2.02-2.65

*significantly different from Test 1 at p <0.05. # significantly different from Test 2 at p <0.05

Tissue sample collection. 48 hours after the final dose effect curve determination approximately 50% of the subjects were deeply anesthetized with an overdose of pentobarbital (>150 mg/kg) and their brains rapidly removed. Brains were flash frozen in an isopentane bath cooled in a dry ice/methanol slurry and stored at -80° C until shipment to UAB. The remaining 50% similarly received an overdose of pentobarbital (>150 mg/kg) and underwent perfusion with 4% formalin.

Table 6. Details of samples prepared for analysis at UAB during Y3 as well as the total collected over Y1 through Y3 combined:

Injury Condition	Chronic Treatment	Preparation	Number Y4	Total Number
Sham injured	Saline	Frozen	0	5
Sham injured	Saline	Perfused	0	5
Sham injured	Oxycodone ED80	Frozen	1	6
Sham injured	Oxycodone ED80	Perfused	0	5
Injured	Saline	Frozen	0	5
Injured	Saline	Perfused	0	5
Injured	Oxycodone ED80	Frozen	3	6
Injured	Oxycodone ED80	Perfused	1	5

5. Tasks performed specific to Aim 1 – evaluation of acute antinociceptive effects of oxycodone and development of tolerance using hotplate response

Studies examining the effect of brain injury on opioid tolerance development using a supraspinally mediated model of acute pain were initiated in Y2. Unlike the WWTW procedure, hotplate nociception/antinociception does not require any habituation to restraint for the procedure. Indeed, as will be discussed below, familiarity with the procedure may contribute to changes observed in baseline responses. All subjects underwent 1 week of acclimation to the laboratory, personnel and handling prior to implantation of iPrecio programmable pumps. Pump implantation was as described for WWTW procedure. On day 5 post-TBI baseline oxycodone antinociceptive effects were determined. Testing began with a SC saline injection (1 ml/kg) followed 15 min later by placement onto a heated metal plate (iiTC Life Science, Woodland Hills, CA) set at 52.5^{o} C. The subjects were confined to the plate by a bottomless clear acrylic enclosure and latency to lick a paw or exhibit escape behavior measured. A cutoff of 40 sec was imposed to avoid tissue damage. All subjects had paws examined repeatedly following testing. No subjects displayed any inflammation or heat associated lesions across the testing days. As with WWTW procedure, pumps were initially filled with saline delivered at 0.2 µl/hour until day 5 post-TBI. Immediately following the initial oxycodone dose-effect curve determination, the saline was extracted from the pump reservoir and it was refilled with either saline or the concentration of oxycodone that would deliver 2.8 mg/kg (determined ED_{80} value) in 30 µl. The oxycodone dose or saline was delivered every 6 hours until 1200 hours on day 19 post-TBI, excluding the 1200 h dose on test

days 11 and 17 post-TBI, at which time subjects were euthanized and brains collected. To summarize, the subjects were slated to undergo the following in order:

1. Habituation to the laboratory and handling.
2. Implantation of iPrecio preprogrammed mini pumps.
3. Sham or lateral fluid percussion injury.
4. Determination of an oxycodone dose-effect curve.
5. Repeated daily dosing with oxycodone or saline via the mini pump.
6. Redetermination of the oxycodone dose-effect curve after 5 days of repeated dosing (day 11 post-TBI).
7. Continue repeated dosing for an additional 5 days.
8. Determine a final oxycodone dose effect curve (day 17 post-TBI).
9. Collect brain tissue for histology and biochemistry at UAB (day 19 post-TBI).

Table 7. Shown are the numbers of subjects assigned to different injury and chronic dosing conditions during Y4 with total numbers for the four years shown in parentheses.

<table>
<tr><td rowspan="6">Total number implanted with infusion pumps = 6 (55)</td><td rowspan="2">Total number undergoing sham injury</td><td colspan="2">Chronic Dosing Assignments</td><td colspan="2">Total completing testing and dosing</td></tr>
<tr><td>Sal</td><td>Oxy ED80</td><td>Sal</td><td>Oxy ED80</td></tr>
<tr><td>= 1 (23)</td><td>0 (10)</td><td>1 (10)</td><td>0 (10)</td><td>1 (11)</td></tr>
<tr><td rowspan="2">Total number undergoing TBI</td><td colspan="2">Chronic Dosing Assignments</td><td colspan="2">Total completing testing</td></tr>
<tr><td>Sal</td><td>Oxy ED80</td><td>Sal</td><td>Oxy ED80</td></tr>
<tr><td>= 5 (32)</td><td>0 (10)</td><td>4 (12)</td><td>0 (10)</td><td>4 (11)</td></tr>
</table>

Results: A comparison of the baseline latencies for all sham controls and brain-injured subjects (Figure 9) shows that **there was no significant difference based on injury condition in responding to the nocicieptive stimulus 5 days post-TBI.** This was consistent with testing in the WWTW procedure. When we extend that comparison out across the three dose-effect curve determinations, we observed a trend in three of the groups to show shorter baselines latencies to respond to the stimulus over time (Figure 10). For the sham controls that received repeated saline, the initial withdrawal latency was longer than observed for the three other groups making this trend seem even stronger. However, even in the latter group the downward effect was not significant. The possible explanations for the change in baseline include: 1) Initial unfamiliarity with the apparatus, testing procedure and potential escape strategies resulting in longer latency

to display overt nociception (licking paw, distress behaviors); 2) coincident with the increasing familiarity with the procedure was anticipation of the exposure to the noxious stimulus resulting in an elevation in anxiety and hyper-responsiveness to the stimulus; and 3) imperceptible tissue damage resulting in actual peripheral hyperalgesia. Based on the observed behaviors of the animals, the frequent examinations of the paws and that testing was performed within a temporal window that should have avoided withdrawal effects, the most probable explanations are a combination of factors 1 and 2. There were also some differences noted in baseline behavior between brain injured and sham controls across treatment conditions however these differences were not consistent nor persistent.

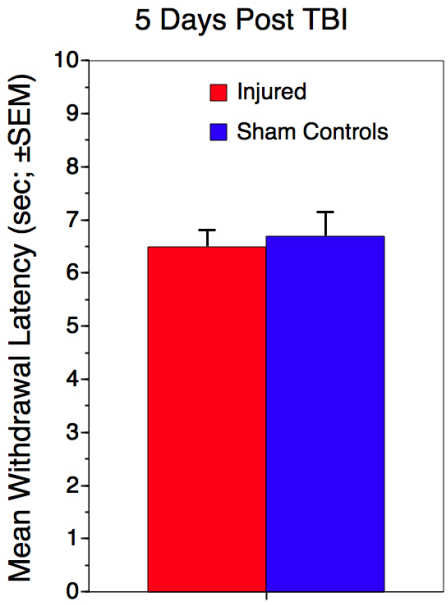


Figure 9. Baseline withdrawal latencies from 52.5°C hotplate surface determined 5 days following moderate TBI (Injured; n=21) or sham control injury (Sham; n=21).

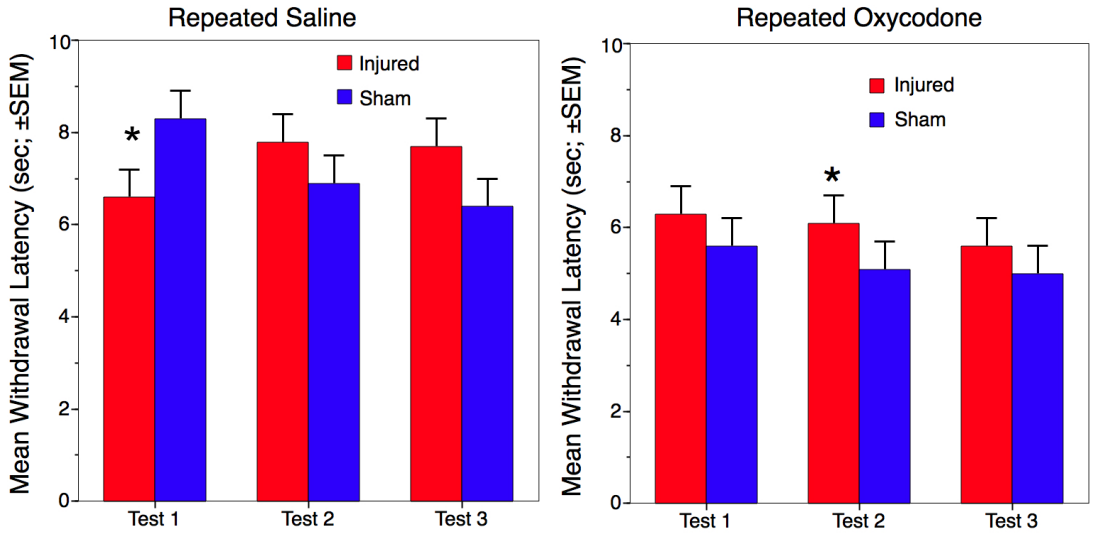


Figure 10. Baseline withdrawal latencies across the three test days (days 5, 11 and 17 post-TBI) for sham controls and moderately injured subjects receiving repeated saline administration (left panel) or 2.8 mg/kg oxycodone (right panel). * Significantly different from sham controls at $p < 0.05$.

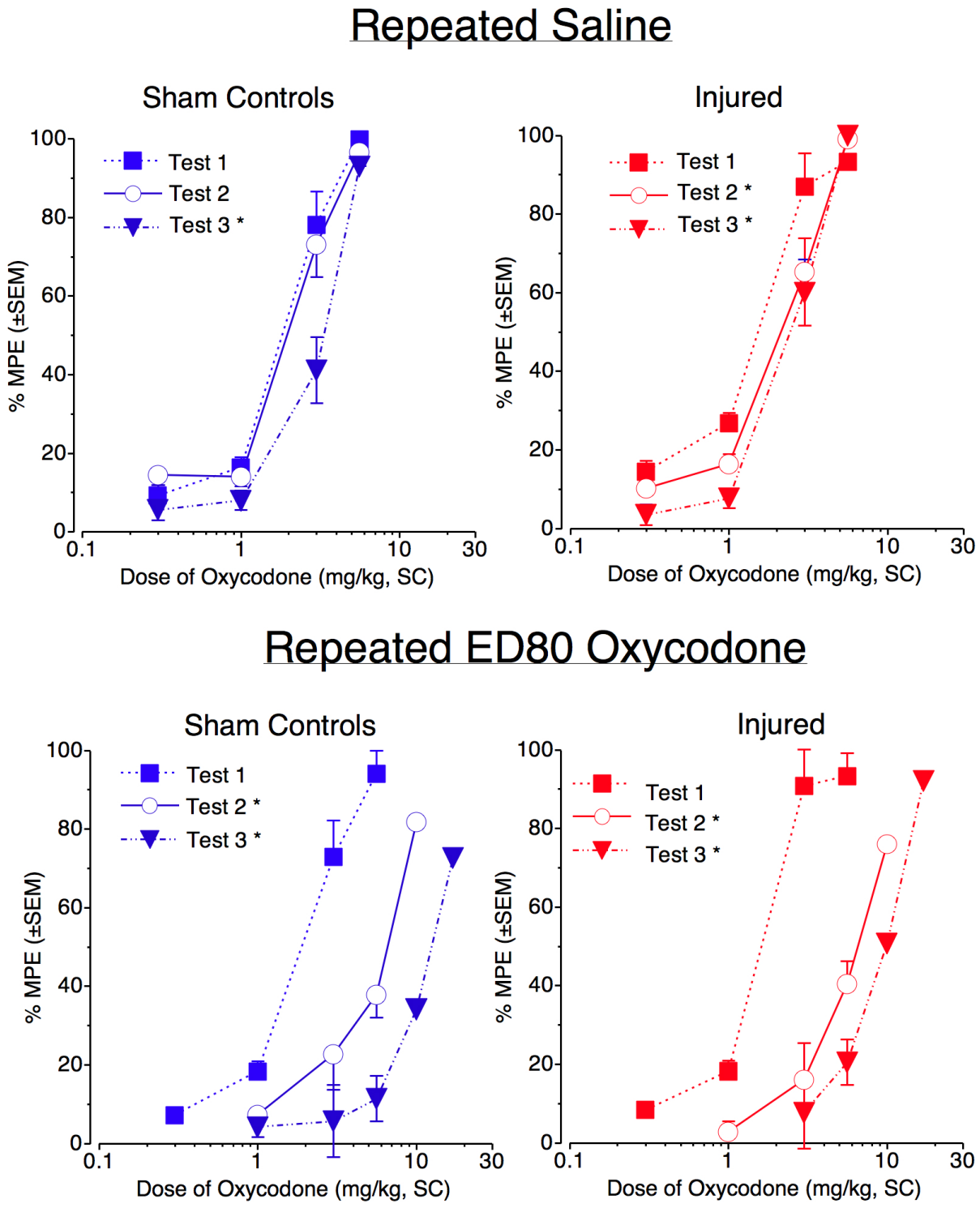


Figure 11. Shown are the percent maximum possible effect for oxycodone antinociceptive effects in the hot plate procedure following repeated dosing with saline (top panels) or 2.8 mg/kg oxycodone (bottom panels) compared within injury condition across the three test days. * significantly different from Test 1 at $p < 0.05$.

For all subjects, oxycodone produced a dose-dependent increase in antinociception (Figure 11 and 12). When comparing the effects of oxycodone across test days in subjects that received repeated saline over the 2-week period, similar to the WWTW procedure we see a modest (< 2-fold), but significant decrease in the potency of oxycodone (Figure 11 and Table 8). This effect was similar across injury condition. When we compared the oxycodone dose effect curves following repeated oxycodone dosing we see a significant, time-dependent rightward shift after both 5 and 10 days of dosing. In contrast to rats receiving saline, the decrease in oxycodone potency was between 5- and 7-fold providing evidence of tolerance development in these subjects. Also similar to the WWTW results, the results across injury condition were virtually identical as illustrated by Figure 12 where the curves for the two injury conditions are almost superimposed. Overall, **there was no difference in the antinociceptive effects of oxycodone between sham controls and brain injured subjects either before or after repeated dosing with oxycodone (2.8 mg/kg) or saline.**

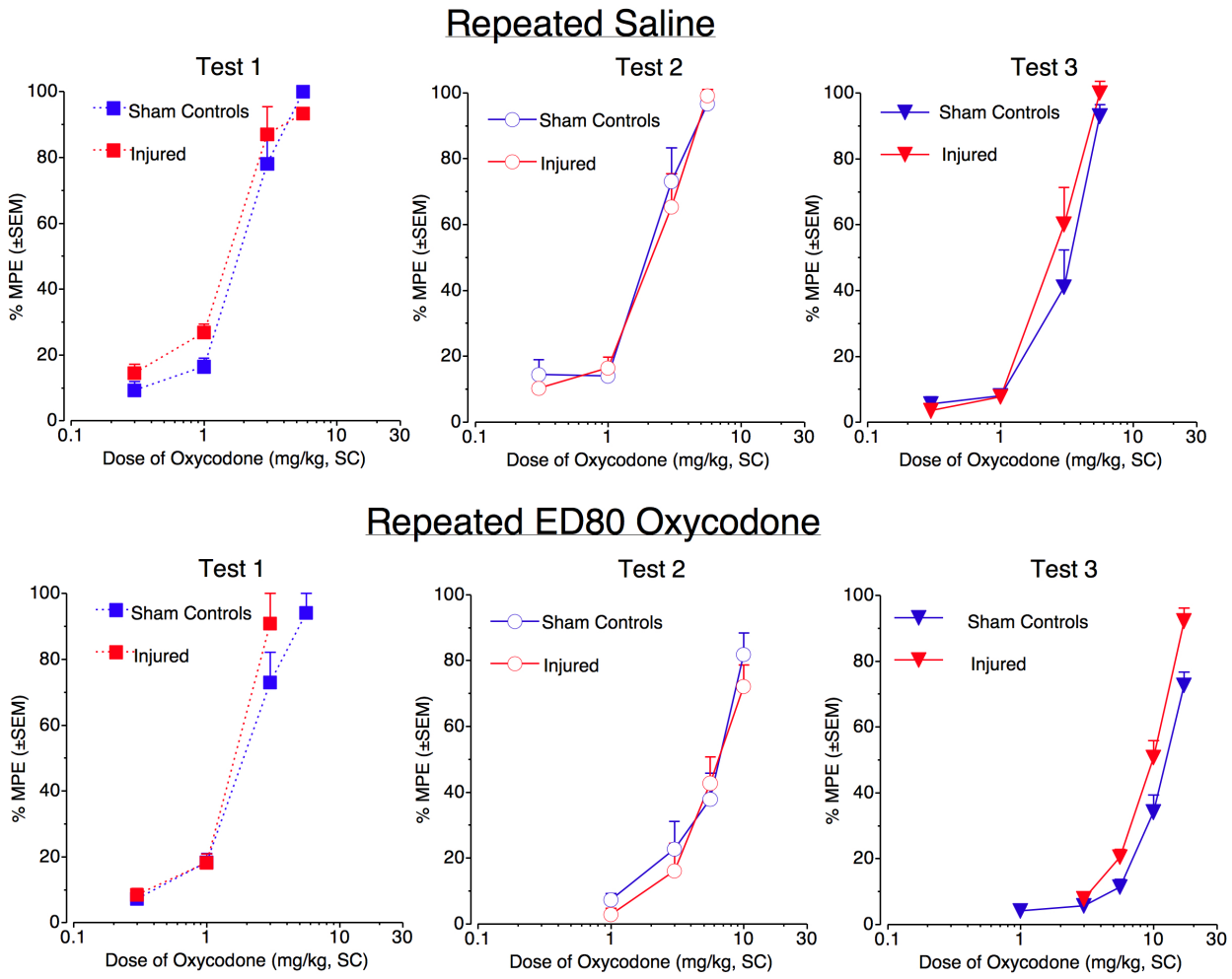


Figure 12. Shown is the percent maximum possible effect for oxycodone antinociceptive effects in the hot plate procedure following repeated dosing with saline (top panels) or 2.8 mg/kg oxycodone (bottom panels) compared across injury condition across the three test days.

Table 8. Shown are the ED_{50} values in mg/kg (±95% C.L.) for oxycodone's antinociceptive effects in a hotplate procedure prior to and after repeated dosing with oxycodone (2.8 mg/kg) or saline.

Repeated Dosing	Injury Group	Test 1 (Baseline) Day 5 Post-TBI	Test 2 Day 11 Post-TBI	Test 3 Day 17 Post-TBI
Saline	Brain-injured	1.21 (0.98-1.50)	2.07* (1.70-2.50)	2.30* (2.00-2.64)
	Sham Controls	1.87 (1.59-2.21)	2.01 (1.64-2.46)	2.61* (1.98-3.44)
Oxycodone ED_{80}	Brain-injured	1.62 (1.47-1.77)	6.57* (5.24-8.23)	8.83* (7.40-10.53)
	Sham Controls	1.74 (1.05-2.88)	5.94* (4.99-7.08)	11.66* (8.52-15.95)

* significantly different from baseline at $p < 0.05$

Tissue sample collection. 48 hours after the final dose effect curve determination approximately 50% of the subjects were deeply anesthetized with an overdose of pentobarbital (>150 mg/kg) and their brains rapidly removed. Brains were flash frozen in an isopentane bath cooled in a dry ice/methanol slurry and stored at -80° C until shipment to UAB. The remaining 50% similarly received an overdose of pentobarbital (>150 mg/kg) and underwent perfusion with 4% formalin.

Table 9. Details of samples prepared for analysis at UAB during Y3:

Injury Condition	Chronic Treatment	Preparation	Number Y3 (Number Y1-4)
Sham injured	Saline	Frozen	0 (5)
Sham injured	Saline	Perfused	0 (5)
Sham injured	Oxycodone ED80	Frozen	1 (6)
Sham injured	Oxycodone ED80	Perfused	0 (5)
Injured	Saline	Frozen	0 (5)
Injured	Saline	Perfused	0 (5)
Injured	Oxycodone ED80	Frozen	3 (6)
Injured	Oxycodone ED80	Perfused	1 (5)

6. Tasks performed specific to Aim 2 assessing the rewarding properties of oxycodone in injured versus sham injured subjects

All studies assessing acquisition of oxycodone self-administration following TBI have been completed. Upon arrival animals were acclimated to handling and the laboratory environment. Five days prior to injury, animals underwent surgical implantation of a chronic indwelling venous catheter under isoflurane anesthesia with morphine pretreatment. A surgical incision was made longitudinally through the skin above the jugular area. The underlying fascia was bluntly dissected and the external jugular vein isolated and ligated with 5-0 silk suture. A

small cut was made into the vein using an iris scissors and the catheter introduced into the vein to a level near but not into the right atrium. The vein encircling the catheter was then tied with 5-0 silk suture. A second suture was used to anchor the catheter to surrounding fascia. The rat was then placed ventral side down on the surgical table and a 2 cm incision was made 1 cm lateral from mid-scapula. A second 0.3 cm incision was then made mid-scapula. The distal end of the catheter was passed subcutaneously from the ventrum (vein cannulation area) to the larger dorsal incision and attached to the cannula/connector. The cannula/connector was then inserted subcutaneously through the larger incision while the upper post portion of the connector/cannula exits through the smaller mid-scapular incision. All incisions were sprayed lightly with a gentamicin/ betamethasone valerate topical antibiotic and the larger dorsal incision and ventral neck incision closed with michel wound clips. Catheters were flushed daily with amoxicillin/sublactam (20/10 mg/kg) in a saline/glycerol/heparin solution to enhance catheter longevity. Periodic infusion of 7.5mg/kg ketamine IV was used to verify catheter patency by presence of immediate onset of sedation.

As shown in Table 10 below, 80 subjects (40 sham controls and 40 injured) have completed testing in the self-administration acquisition procedure. This includes evaluation of acquisition at the 3 doses of oxycodone originally proposed and the additional dose amended to the SOW in July 2013. Five days following TBI or sham injury described above, subjects began daily self-administration testing conducted in standard operant chambers housed within isolated and ventilated enclosures (Med Associates). Each chamber was equipped with two response levers with a white stimulus light above each lever, a 5-watt house light in the rear wall and an adjustable Sonalert (Model ENV-223AM, Med Associates) in the upper left wall. During each session, infusion tubing, protected by a stainless steel spring tether (Plastics One), was connected to the back-mounted cannula pedestal. Infusions were delivered via a peristaltic pump located outside each chamber. Schedule parameters were controlled by MED-PC IV software (Med Associates) running on a PC compatible computer. Rats were brought to the laboratory daily (7 days/week) and allowed to acclimate for 15-30 min before being connected to the infusion tether and placed in the chamber for the 2-hour acquisition session. During the session, a single response, fixed ratio (FR) 1, on the right lever resulted in the delivery of a 0.1-ml, 3-sec infusion of one of the three oxycodone doses as shown in Table 10. Responding on the left lever had no scheduled consequence but was recorded as a measure of behavioral activity in the chamber. Criteria for acquisition was three consecutive days of receiving > 15 infusions and responses on the active lever > responses on the inactive lever. Subjects were permitted up to 21 sessions to achieve criteria.

In Y3, 25 subjects began acquisition testing and 20 completed testing, the remaining 5 (2 sham, 3 injured) lost catheter patency prior to 21 days. Overall, we have now completed acquisition testing at 0.003, 0.01, 0.03 and 0.056 mcg/kg/infusion oxycodone doses. Of these subjects, 10 (5 injured, 5 sham) failed to acquire self-administration behavior. As expected, availability of 0.03 and 0.056 mg/kg/infusion doses resulted in the highest percent acquisition in both the injured (100%) and sham control subjects (90 - 100%). The lowest dose, 0.003

mg/kg/infusion, resulted in acquisition levels (50% of injured and 60% of sham controls) relatively consistent with our predicted range of 40-50%. Availability of the intermediate dose of oxycodone was associated with a significant difference in acquisition levels between the injured rats and sham controls with the injured animals demonstrating 100% acquisition versus 60% for the controls (Figure 13A). What did not show considerable difference between the infusion doses was the mean rate of acquisition as shown in Figure 13B. Analysis of the daily cumulative acquisition percent for the sham and injured subjects as shown in Figures 14 and 15 provides additional insight into the patterns for acquisition and reaffirms the difference in behavior between the sham controls and the brain-injured subjects at the intermediate dose. **This difference suggests that the brain-injured subjects are more sensitive to the reinforcing/rewarding effects of oxycodone.**

Table 10. Shown are the numbers of subjects assigned to different injury and dosing conditions across Y4 with total numbers for Y1 through Y4 combined in parentheses.

Number catheterized	Number undergoing sham injury	Number entering acquisition	Number completing acquisition	Oxycodone Dose (mg/kg/infusion)			
				0.003	0.01	0.03	0.056
= 0 (126)	= 0 (64)	=0 (51)	= 0 (40)	0 (10)	0 (10)	0 (10)	0(10)
	Number undergoing TBI	Total entering acquisition procedure	Total completing acquisition assessment	Oxycodone Dose (mg/kg/infusion)			
				0.003	0.003	0.003	0.056
	=0 (72)	= 0 (48)	= 0 (40)	0 (10)	0 (10)	0 (10)	0(10)

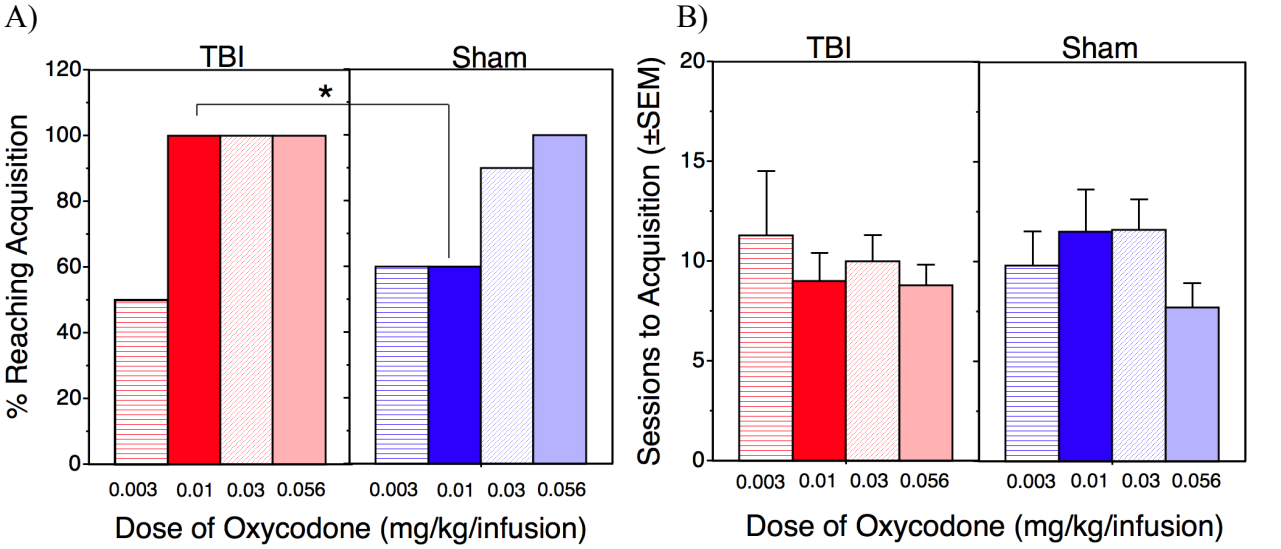


Figure 13. Panel A) Shown are the percent of brain-injured (TBI) and sham control subjects acquiring self-administration behavior across the different oxycodone doses. (n=40 for sham controls, n=40 for brain-injured subjects). * significantly different from sham controls at $p < 0.05$. Panel B) Shown are the mean number of days to achieve self-administration acquisition criteria for sham and brain-injured (TBI) subjects across the four oxycodone doses.

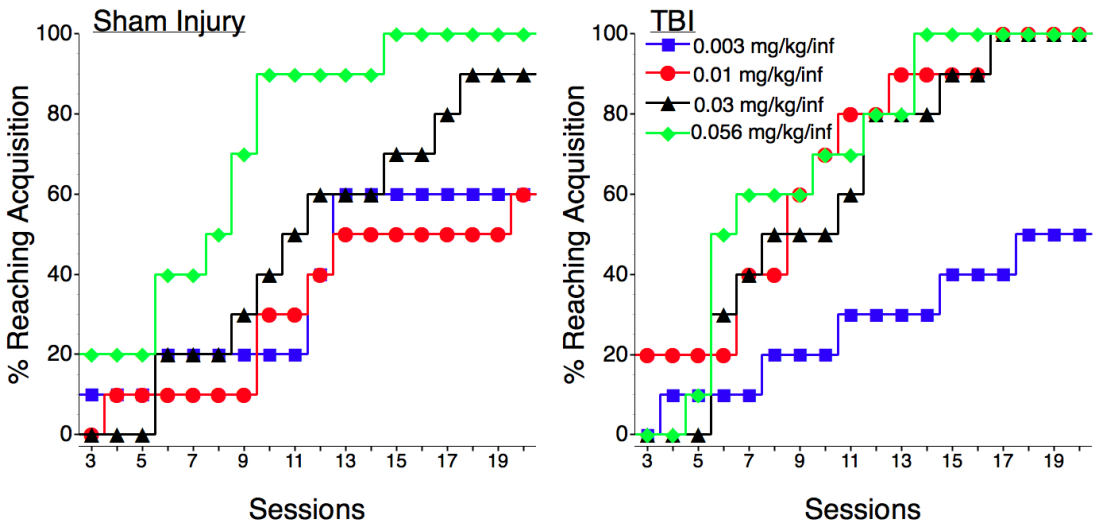


Figure 14. Shown are the cumulative percentages of subjects acquiring self-administration behavior across sessions for sham controls (left panel) and brain-injured (right panel) rats for each of the four oxycodone doses.

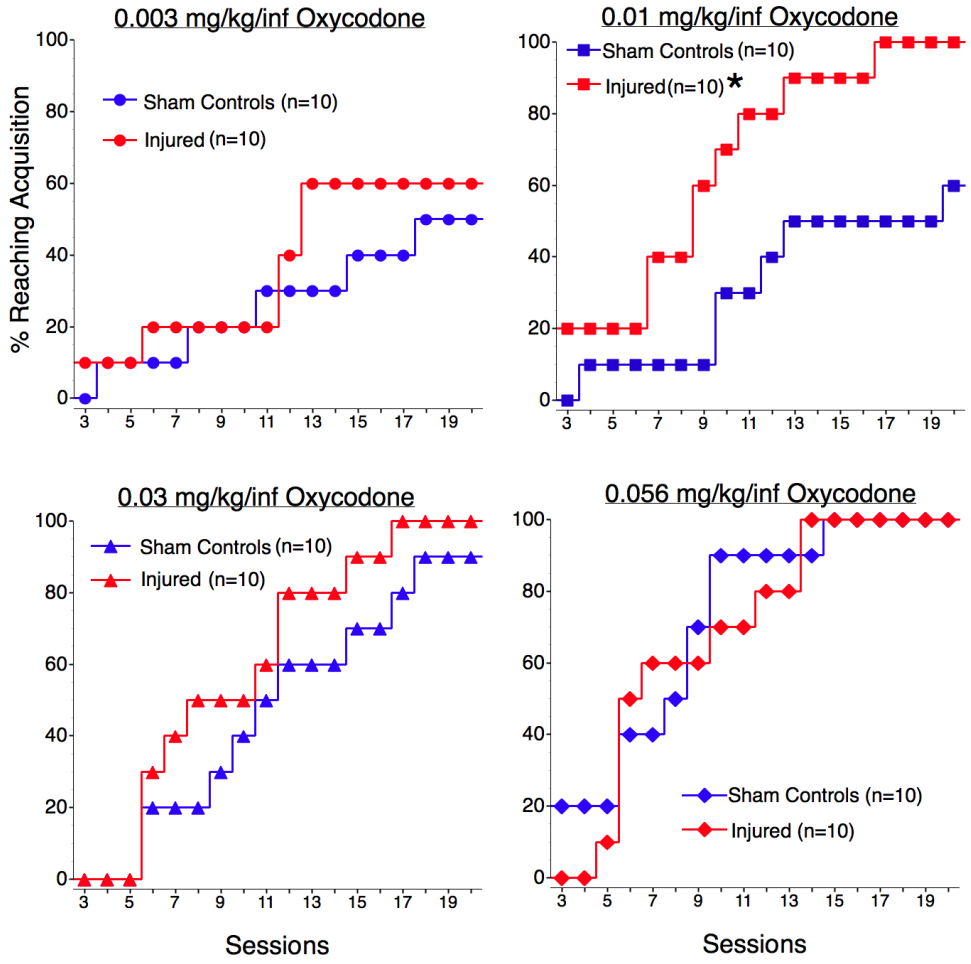


Figure 15. Shown are the cumulative percentages of subjects acquiring self-administration behavior across sessions for sham control and brain-injured rats for each of the four oxycodone doses.
* significantly different from sham controls at $p < 0.05$.

Following acquisition, the rats were allowed to continue self-administering the acquisition dose until performance was stable. Stability was defined as 3 days in which the number of infusions did not differ from the mean for the 3 days by more than 25% with no increasing or decreasing trends. The mean number of infusions across those three days is shown in Figures 16 and 17. For both groups, responding on the active lever (Figure 16) was significantly greater than inactive lever responding. The latter serves as a measure of general activity within the chamber. That responding on the inactive lever was significantly lower across all oxycodone doses in both demonstrates that oxycodone was serving as a positive reinforcer of behavior in both the injured rats and sham controls and that responding on the active lever was not simply by chance but was goal-directed. For the brain-injured subjects, there was an inverted U-shaped dose-effect curve relating dose per infusion to infusion rate. This type of dose-effect relationship is typical of drugs effective as positive reinforcers (Young and Herling, 1986). For the sham control subjects there minimal dose-dependent changes in infusions were observed across the four oxycodone doses however the total number of infusions was greatest for the 0.01 mg/kg/infusion dose. When comparing across injury condition, intake at the 0.03 mg/kg/ infusion dose was significantly greater in the brain-injured subjects (Figure 17). There was also some evidence of this effect at the 0.056 dose as well, however the difference between injury conditions was not significant. Taken alone, these data might suggest that the reinforcing effects of oxycodone were less potent in the brain-injured subjects requiring higher intake to achieve intoxication, however the acquisition data suggest the opposite. **Alternatively, the data could suggest that the brain-injured subjects are less sensitive to the use-limiting effects of the oxycodone (sedation, dysphoria, etc) and will self-administer a greater total dose of oxycodone compared to sham control subjects.** In humans this propensity to take more drug and higher total doses of drug has been posited to increase risk for escalating drug use and addiction (de Wit and Phillips, 2012).

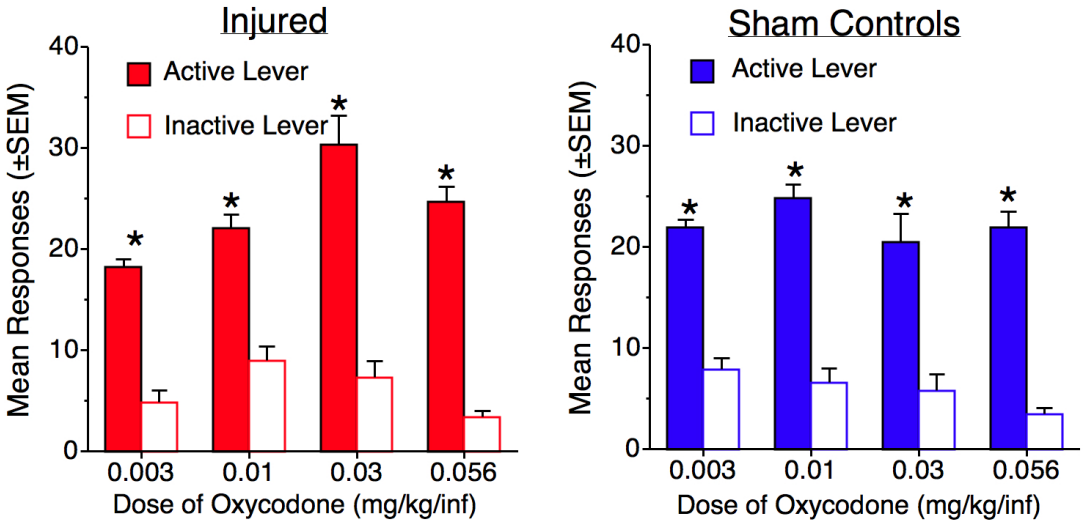


Figure 16. Shown are the mean number of lever responses emitted on the active (closed bars) and inactive (open bars) levers during three days of stable responding for oxycodone self administration for sham controls (left panel) and brain-injured (right panel) rats. * significantly different from inactive lever responding at $p < 0.05$.

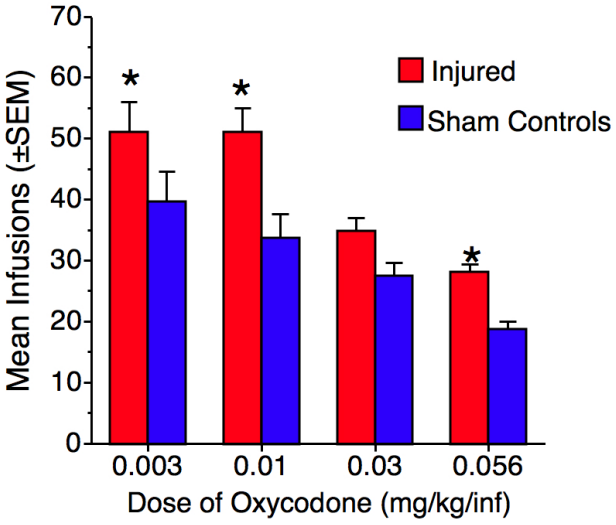


Figure 17. Shown are the mean number of oxycodone infusions self-administered across three days of stable responding comparing levels for sham controls to brain-injured rats across the four acquisition doses. * significantly different from sham controls at $p < 0.05$.

Different behavioral effects of oxycodone may have different relative potencies. Even though we did not see differences in potency in our analgesia models, this does not preclude differences in reinforcing potency or sedative potency based on presence or absence of injury. At higher doses, opioid drugs produce sedative effects in addition to analgesic effects resulting in motor impairment. However, at low and intermediate doses, opioids can actually increase locomotor activity due to disinhibition of neural circuits associated with dopamine release. To further investigate the relative potency of oxycodone for producing stimulatory and sedative effects, separate groups of injured and sham control subjects were evaluated for activity levels following administration of different doses of oxycodone in an open field. This procedure helped assess the role of behavioral activity in the differences in response levels noted between the two injury conditions.

A total of 24 rats underwent lateral fluid percussion injury (n=13) or sham injury (n=11) procedures following approximately 2 weeks acclimation to handling and the laboratory. Of these subjects, 20 continued in the study; one died during the craniectomy procedure, two died during the injury procedure and one received an inadequate level of injury to qualify for inclusion in the study. Beginning 5 days after TBI or sham injury, all subjects were placed in an open field chamber (41cm X 41 cm X 20 cm) equipped with 16 photobeam cells (ENV15, MedAssociates) and allowed to ambulate freely for 30 min. Total distance travelled and location within the field were determined based on photobeam breaks which were recorded and analyzed using MedPC software (MedAssociates, St. Albans, VT). This initial session permitted collection of basal activity levels as well as habituated the rats to the environment and the procedure. Subsequently, every 4 days the process was repeated however rats received either an injection of saline or one of three doses of oxycodone (0.3, 1.0 or 1.7 mg/kg) administered SC 15 min prior to placement in the open field. Saline and oxycodone doses were administered in a counterbalanced design in order to mitigate any order effects. As shown in Figure 18 there was no difference in distance travelled following saline administration. In addition, at the 0.3 mg/kg dose of oxycodone, both brain injured and sham control rats displayed a modest increase in activity levels relative to saline, although this increase was not significant. While both groups exhibited a dose-dependent decrease in activity as the dose was raised, there was a trend for activity levels to remain higher in the brain-injured subjects relative to the controls, particularly

at the 1.7 mg/kg dose. However, this difference did not reach significance. Regardless, this does suggest a possible **decreased sensitivity to the depressant effects** of the oxycodone which would be consistent with the higher level of intake seen in the self-administration procedure at the 0.03 and 0.056 mg/kg/infusion doses.

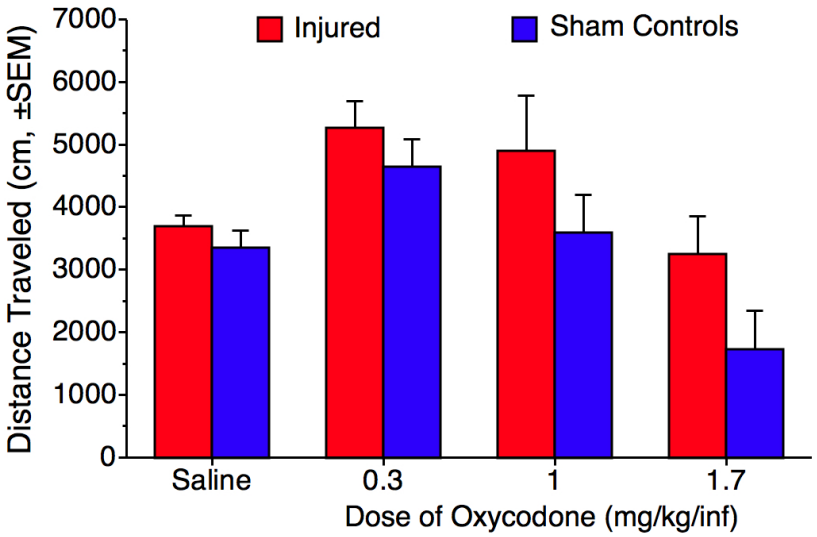


Figure 18. Shown are the mean distances travelled in an open field for sham control and brain injured subjects following administration of saline or different doses of oxycodone.

The primary dependent measures collected during acquisition testing included the number of days to meet the acquisition criterion, the percentage of rats per group meeting the criteria, and the number of infusions received following stabilization of responding. However, data regarding a number of other parameters were also routinely collected. Another measure that was analyzed was responding which occurred on the active lever during the 1-min timeout that was imposed following each infusion. During the 3-sec infusion of drug solution, the houselight was extinguished and the stimulus light over the lever was illuminated. When the infusion was complete, the stimulus light was also extinguished and the operant chamber remained unlit for the 1-min timeout (TO) period. Responding was recorded but did not result in delivery of drug or any light response. At the end of the TO, the houselight was once again illuminated and responding on the active lever resulted in drug presentation. As shown in Figure 19, the brain-injured subjects elicited a significantly greater number of responses during the TO period than the sham controls during availability of the 0.03 and 0.056 mg/kg/infusion doses of oxycodone. Timeout responding has been viewed as a potential measure of drug seeking however others perceive it as a measure of impulse control – can the subject wait for the timeout to end and the active period to begin before responding again. Either explanation could account for what was seen in our study. Therefore we also examined responding for food reward – a nondrug reinforcer of behavior - to determine whether the greater TO responding was reflective of drug seeking or due to generalized dysfunction of impulse control. The same

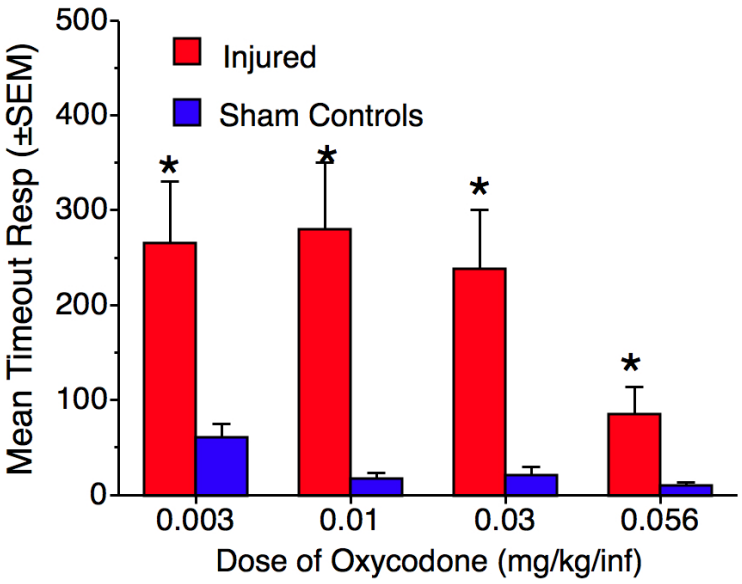


Figure 19. Shown are the mean number of lever responses emitted on the active lever during the timeout period over the three days of stable responding for oxycodone self administration for sham controls and brain-injured rats. * significantly different from sham controls at $p < 0.05$.

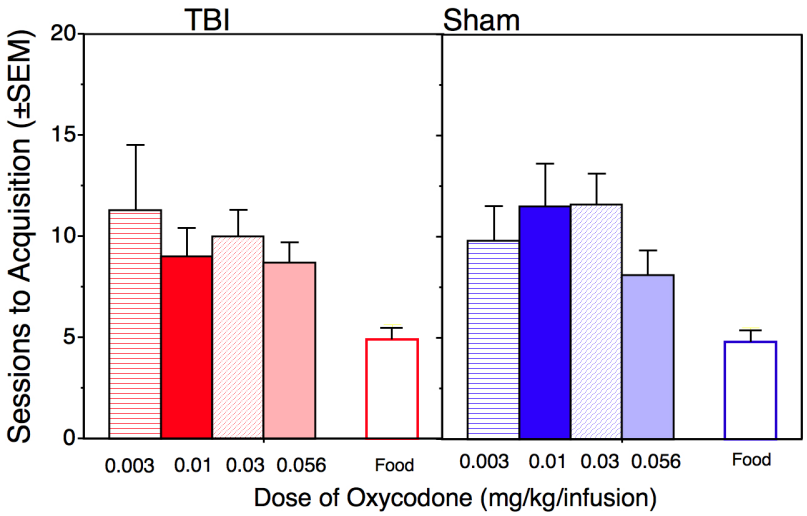
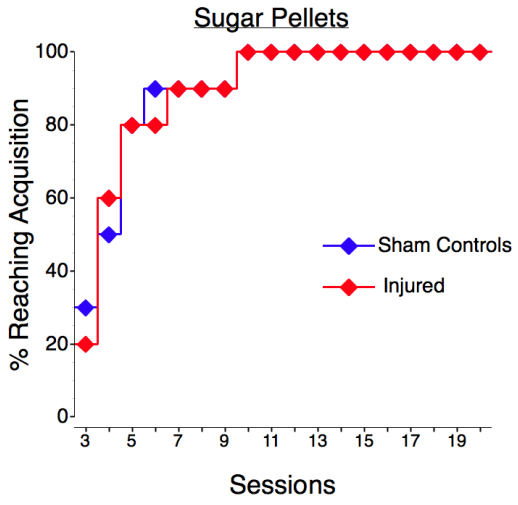


Figure 20. Shown are the mean number of days to achieve acquisition criteria for oxycodone self-administration and food maintained behavior for sham and brain-injured (TBI) subjects.

Figure 21. Shown are the cumulative percentages of subjects acquiring food-maintained responding behavior across sessions for sham control and brain-injured rats.



subjects that were evaluated for locomotor activity were used for this component of the project. All subjects were evaluated for acquisition and maintenance of food maintained responding during daily 1-hour sessions in the morning. Every 4th day, minimally 3 hours after the operant

session, rats were evaluated for locomotor response to oxycodone or saline. The operant session conditions were the same as during the oxycodone self-administration sessions with the exception that the session was only 1 hour in duration and each response on the active lever resulted in presentation of a 45-mg grain based food pellet (BioServe, Frenchtown, NJ). There was no difference in percent or rate of acquisition between sham controls and brain-injured subjects (Figures 20 and 21). All subjects, regardless of injury condition, acquired food maintained responding. In addition, they acquired the behavior very rapidly with 50-60% reaching acquisition criteria within 4 days. Interestingly, this is faster acquisition than that for oxycodone. As shown in Figure 22, once responding stabilized, brain-injured subjects responded for a small but significantly greater number of pellets during the sessions. However, unlike responding for oxycodone, food maintained responding was not associated with a greater level of time-out responding. This suggests that **the perseverative behavior exhibited during oxycodone self-administration is not simply a generalized loss of impulse control but rather due to greatly increased drug seeking in brain-injured subjects.**

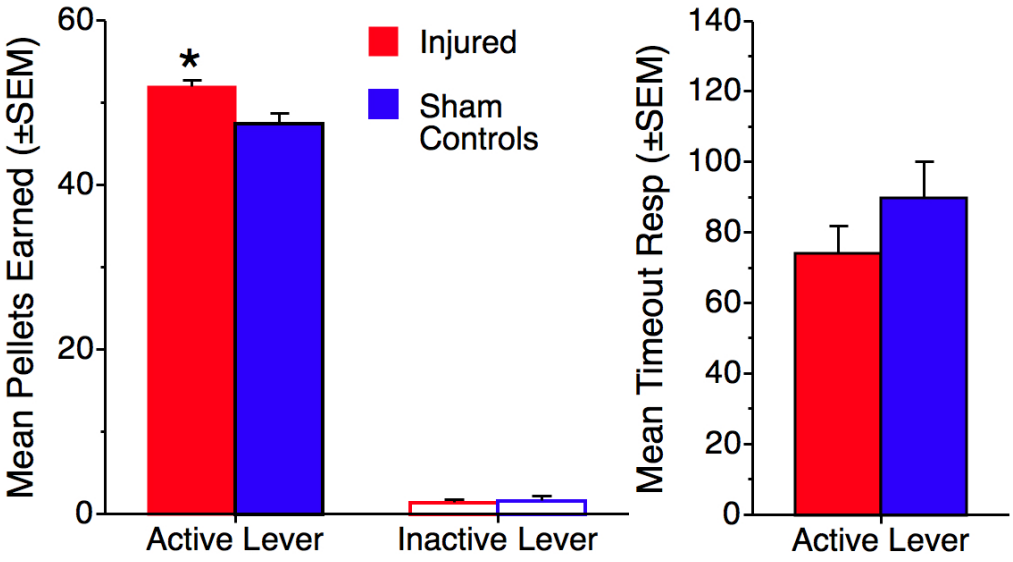


Figure 22. Shown in the left panel are the mean number of lever responses emitted on the active (closed bars) and inactive (open bars) levers during three days of stable responding for 45-mg pellets for sham controls and brain-injured rats. In the righthand panel, shown are the mean number of lever responses emitted on the active lever during the timeout period over the three days of stable responding for 45-mg pellets for sham controls and brain-injured rats.

Tissue sample collection. Minimally 72 hours after the final self-administration session, approximately 50% of the subjects were deeply anesthetized with an overdose of pentobarbital (>150 mg/kg) and their brains rapidly removed. Brains were flash frozen in an isopentane bath cooled in a dry ice/methanol slurry and stored at -80° C until shipment to UAB. The remaining

50% similarly received an overdose of pentobarbital (>150 mg/kg) and underwent perfusion with 4% formalin.

Table 11. Details of tissue samples collected for analysis at UAB in Y3 (Y1 through Y3 combined in parentheses).

Behavioral Group	Injury Condition	Preparation	Number
Oxycodone self-ad acquisition	Sham injured	Frozen	5 (20)
	Sham injured	Perfused	5 (20)
Food acquisition/ Locomotor activity	Sham injured	Frozen	5 (5)
	Sham injured	Perfused	5 (5)
Oxycodone self-ad acquisition	Injured	Frozen	5 (20)
	Injured	Perfused	5 (20)
Food acquisition/ Locomotor activity	Injured	Frozen	5 (5)
	Injured	Perfused	5 (5)

7. Tasks performed specific to Aim 2 assessing relapse-like behavior following oxycodone self-administration in injured versus sham control subjects

A total of 22 subjects completed testing of reinstatement behavior in order to assess the effect of moderate brain injury on relapse-like behavior. Subjects were prepared with indwelling intravenous catheters and underwent sham or moderate injury procedures as described for the acquisition study. Based on findings in the acquisition study, the dose of 0.03 mg/kg/infusion was selected for use in the reinstatement studies. Therefore all subjects assigned to reinstatement assessment began training in self-administration of 0.03 mg/kg/infusion of oxycodone on day 5 post-TBI as outlined in Table 12. Unlike the acquisition procedure, any subject not demonstrating minimally 10 infusions/session by day 3 of training was primed (received an infusion through investigator initiated responding) and also had the lever baited with peanut butter to promote responding. Of the 25 rats that entered reinstatement training/testing, 1 subject failed to acquire the behavior within the allotted 14-day period (sham control rat). As soon as subjects met acquisition criteria and reached stable levels of responding as described above for acquisition, they entered extinction training. During extinction sessions, subjects were placed in the operant chambers and connected to the tethers. The behavioral session was initiated as signaled by illumination of the houselight and presentation of the response levers. However, responding on either lever had no scheduled consequence – no drug infusion, no extinguishing of the houselight, no stimulus light presentation. Subjects were given up to 21 days of extinction training to meet extinction criteria. To be considered to have extinguished, the number of total responses emitted by each subject had to be less than 50% of their baseline oxycodone administration level and responding over three extinction days had to be within 25% of the mean of the three days with no trends. Two subjects failed to meet extinction criteria within the 21-day time window, one sham control and one brain injured subject. The remaining 22 subjects met extinction criteria, and for most, particularly the brain-injured subjects, responding was well below 50% of control levels by the time stability was achieved. Once extinction criteria were met, subjects underwent prime- and cue–induced

resinstatement of responding. All subjects underwent both reinstatement conditions with minimally 3 and up to 7 days of renewed extinction training in between the sessions (until extinction responding was once again stable). Prime- and cue-induced sessions were counterbalanced across subjects within each injury condition to compensate for any order effects. During cue-induced reinstatement sessions, the initial response on the active lever resulted in presentation of the drug-associated cues: houselight extinction, stimulus light presentation and 1-min timeout period followed by return to extinction session conditions for all subsequent responses. During prime-induced reinstatement sessions, 1 mg/kg oxycodone was administered SC 15 min prior to session onset otherwise the prime reinstatement behavioral session was identical to the extinction session.

Table 12. Shown are the numbers of subjects assigned to different injury and dosing conditions across Y3 with totals for the 3 years in parentheses.

Number catheterized	Number undergoing sham injury	Number entering Reinstatement procedure	Number completing Reinstatement procedure
0 (30)	=0(12)	=0(12)	=0(10)
	Number undergoing TBI	Number entering Reinstatement procedure	Number completing Reinstatement procedure
	=0(18)	=0(13)	=0(12)

Figure 23 presents reinstatement responding for sham controls and injured subjects. Unlike the acquisition procedure, responding for oxycodone under baseline conditions was modestly but significantly lower for the brain-injured subjects compared to the sham controls. This is likely due to the more rapid self-administration training and more limited total time of oxycodone exposure during reinstatement training compared with acquisition. Whereas subjects in the reinstatement procedure had acquired the behavior and reached stability within 14 days, acquisition subjects were permitted up to 21 days (mean time was approximately 10 days) with additional access permitted for reaching stability. This meant many subjects in the acquisition study continued self-administering oxycodone for up to 30 days total, over twice as long as many of the reinstatement subjects. During that time, responding in the brain-injured subjects continued to increase modestly resulting in the higher final intake for brain-injured versus sham controls seen in the previous study. For the relapse study subjects, it required an average of 10.1 and 6.6 days for injured and sham control subjects to reach extinction criteria, respectively (Figure 24). This suggests, similar to the timeout responding noted in the animals evaluated for acquisition behavior, the TBI subjects were more persistent in their responding. The significantly slower extinction could be due to stronger drug seeking in the brain injured subjects. Alternatively the slow extinction rate could reflect cognitive impairment that is commonly noted following TBI. Basically, the injured subjects may have required more sessions to learn NOT to respond for drug. Surprisingly, once behavior was extinguished however, injured subjects exhibited significantly lower responding during extinction sessions (see Figure 23, “Extinction” values). Baseline extinction responding was calculated based on

the mean responses emitted during the 3 extinction training sessions preceding the prime- and the cue-induced reinstatement sessions (6 sessions total/subject). As can be seen (Figure 23), even the single cue presentation resulted in a significant increase in responding by both sham controls and brain-injured subjects, although the relative increase in responding was significantly less in the brain-injured subjects compared to sham controls. This is consistent with numerous studies examining relapse-like behavior in both preclinical and clinical studies (Shaham et al., 2003; Crombag et al., 2008; Smith and Aston-Jones, 2012). Indeed, the effect of cues has proven to be a significant cause of relapse in human substance abuse and has been shown to maintain high levels of responding following reinstatement for psychostimulants and opioids in preclinical studies. Unlike the cue, the 1 mg/kg oxycodone prime (Figure 23) did not result in a significant increase in levels of responding relative to baseline for either brain-injured or sham controls (no reinstatement of behavior). The dose selected was based on responses in the antinociception testing and in the literature. Indeed the 1 mg/kg dose in antinociception testing did not appear to cause overt sedation/motor suppression nor did it suppress activity relative to controls in the open field tests, however approximately half of the first ten subjects tested were obviously intoxicated at this dose and exhibited few if any responses during the behavioral session. The depressant effects of the oxycodone prime was even more pronounced in the brain-injured subjects and actually resulted in a significant decrease in responding. This is in complete contrast to what was seen in the open field behavior. However, given this confound to our reinstatement procedure, we also tested a subset of subjects with 0.3 mg/kg dose of oxycodone administered subcutaneously 15-min prior to the session. The data for this subset, including their extinction baseline data are shown in Figure 25. For these subjects, there was no significant difference in levels of extinction behavior based on injury condition. Following the 0.3 mg/kg oxycodone prime, both groups showed modest (brain-injured) to moderate (sham control) increases in responding during the session. While significantly different from each other, given the relatively small group sizes, the inherent variability and the modest effect in the brain-injured subjects, neither group's prime-induced responding was significantly different from their respective extinction baseline levels. Evaluation of the prime-induced reinstatement data normalized for individual baseline rates (expressed as a percent of control), did not change the statistical comparison.

Overall, the data suggest that the brain-injured subjects exhibit impaired learning and cognitive inflexibility in terms of stopping drug intake. First, **the injured subjects required longer periods of extinction training to discontinue responding than the sham controls. This suggests that TBI patients that develop opioid abuse may have a more difficult time stopping drug use than uninjured opioid abusers. However, once behavior has been extinguished, there appeared to be a greater reinstatement in the sham controls relative to injured subjects in both the prime- and cue-induced reinstatement procedures. Positively,**

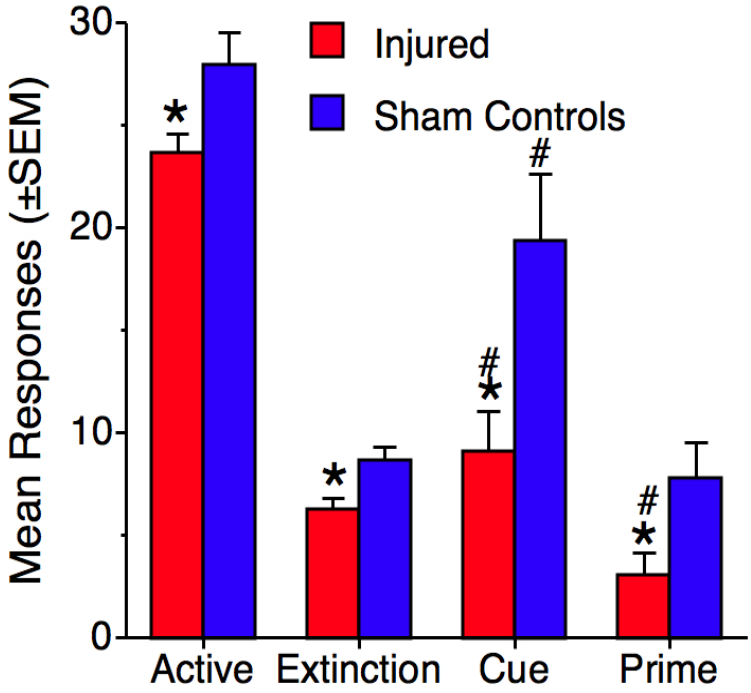


Figure 23. Shown are the mean number of responses on the active lever during oxycodone reinforced sessions (Active), following extinction training (Extinction), during cue-induced (Cue) and prime-induced reinstatement for sham controls (n=10) and brain-injured (n=12) rats. * significantly different from control at $p < 0.05$. # significantly different from extinction baseline at $p < 0.05$.

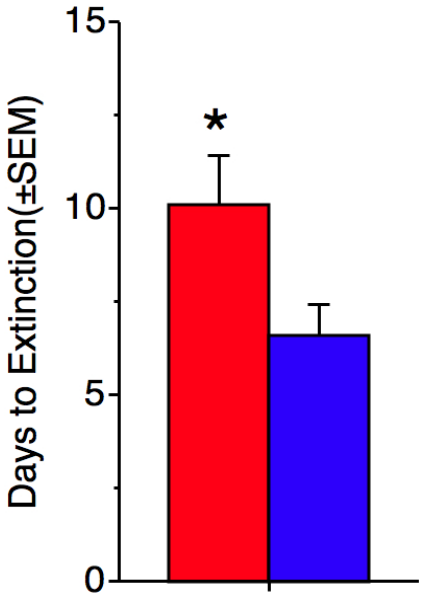


Figure 24. Shown are the mean number of days required to reach extinction criteria for sham controls (n=10) and brain-injured (n=12) rats. * significantly different from control at $p < 0.05$.

this suggests a lower propensity for relapse in brain-injured subjects once they have become abstinent. Before accepting the latter conclusion however, several additional factors must be considered. For one, it may simply be a dose-associated difference between the two injury groups. If the injured subjects are exhibiting a greater level of rate suppression due to the oxycodone, testing lower doses of oxycodone may uncover higher levels of reinstatement in the injured subjects. Additionally, our procedure utilized active extinction training (learning to stop responding) whereas many humans that abuse opioids will undergo abstinence without extinction learning which could impact the risk for relapse. Finally, as discussed earlier in this section, these subjects had limited exposure to oxycodone and were not physically dependent. It is not within the scope of the current grant to address these additional questions however they will be included in future grant submissions exploring additional aspects of post-TBI substance abuse to ensure a full understanding of the risks of relapse in brain-injured subjects.

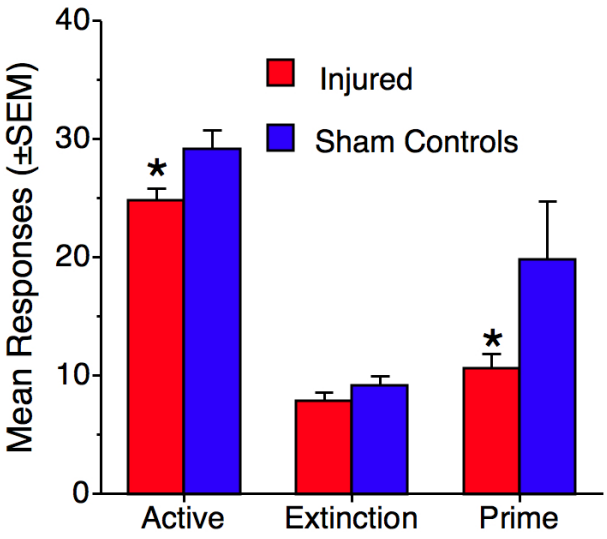


Figure 25. Shown are the mean number of responses on the active lever during oxycodone reinforced sessions (Active), following extinction training (Extinction)and during 0.3 mg/kg oxycodone prime-induced reinstatement for sham controls (n=5) and brain-injured (n=5) rats. * significantly different from extinction baseline at $p < 0.05$.

Tissue sample collection. Minimally 72 hours after the final extinction session, approximately 50% of the subjects were deeply anesthetized with an overdose of pentobarbital (>150 mg/kg) and their brains rapidly removed. Brains were flash frozen in an isopentane bath cooled in a dry ice/methanol slurry and stored at -80° C until shipment to UAB. The remaining 50% similarly received an overdose of pentobarbital (>150 mg/kg) and underwent perfusion with 4% formalin.

Table 13. Details of tissue samples prepared and sent to UAB. Numbers in parentheses represent total for 3 years.

Injury Condition	Preparation	Number
Sham injured	Frozen	0 (5)
Sham injured	Perfused	0 (5)
Injured	Frozen	0 (6)
Injured	Perfused	0 (6)

8. Tasks performed specific to Aim 2 assessing rewarding strength of oxycodone in injured versus sham injured subjects

Acquisition of self-administration behavior and maintenance intake under FR schedules provide information on the presence or absence of reinforcing effects (does a drug solution maintain IV self-administration or not) as well as the relative potency of different doses in producing those effects. However, it does not provide information about the relative strength of that drug solution to function as a reinforcer/reward. A complimentary method used to assess the reinforcing strength of drugs is the progressive ratio (PR) procedure. Under a PR schedule of self-administration, the number of lever-presses required to receive each subsequent drug infusion is increased with the final ratio value completed by a subject referred to as the "break point". Increases in break points have been interpreted as enhanced drug seeking behavior and a higher relative reinforcing efficacy (i.e., reinforcing strength). PR schedules have been useful in

detecting differences between experimental groups, such as age, stress conditions or sex, for the same drug reinforcer (Richards and Roberts, 1996; Rowlett, 2000; Stafford et al., 1998). Similarly we have used this approach to evaluate the reinforcing strength of drugs in rats following induction of TBI.

Subjects were prepared with indwelling intravenous catheters and underwent sham or moderate injury procedures as described for the acquisition study (Section 6). Based on findings in the acquisition study, the dose of 0.03 mg/kg/infusion was selected for use as the training and baseline dose off which to test other oxycodone concentrations. All subjects allocated to the PR study began training in self-administration of 0.03 mg/kg/infusion of oxycodone on day 5 post-TBI as outlined in Table 14. Unlike the acquisition procedure, any subject not demonstrating minimally 10 infusions/session by day 3 of training was primed (received an infusion through investigator initiated responding) and also had the lever baited with peanut butter to promote responding. All subjects that entered training/testing acquired the behavior within the allotted 14-day period. Once reaching acquisition criteria, the animals continued to run in daily sessions until demonstrating stable behavior defined as 3 consecutive sessions with no more than 25% change in the number of infusions delivered with no increasing or decreasing trends in numbers. After reaching stability, the animals were tested using a substitution procedure where different concentrations of oxycodone (0.003, 0.01 and 0.056 mg/kg/infusion) were substituted for the 0.03 mg/kg/infusion training dose. Each dose was tested for 11 consecutive sessions. Maintenance performance under FR 1 conditions was assessed for 4 days followed by testing under a PR schedule for 7 consecutive test sessions. During the PR sessions, the number of active lever-presses required to receive each infusion incremented as follows: 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, 219, 268, 328, 402, 492, 603, 737. Mean data from the last three of the four FR sessions and of the seven PR sessions was used in the analysis. Following completion of the PR analysis for an individual dose, the subject was returned to baseline conditions – 0.03 mg/kg/infusion oxycodone solution available under FR1 schedule - until responding was stable. Then, an alternate dose of oxycodone was evaluated first under FR1 and then PR conditions. This procedure continued until all doses were tested or subjects no longer had patent catheters. Dose presentation was counterbalanced to minimize order effects.

Table 14. Shown are the numbers of subjects assigned to different injury conditions across Y1-Y3 for assessment of reinforcing strength using a progressive ratio schedule.

Number catheterized	Number undergoing sham injury	Number entering PR procedure	Number completing PR procedure
33	=15	=14	=10
	Number undergoing TBI	Number completing PR procedure	Number completing PR procedure
	=18	=14	=10

A total of 33 subjects were entered into the protocol towards completing this component of Aim 2. Because of the extended testing time, some subjects did not complete all test doses (average time to complete testing three doses was 80 days) within the life span of two IV catheters (right and left jugular veins were catheterized sequentially). Therefore 14 sham and 14 brain-injured subjects underwent testing in order to complete testing of each dose in 10 subjects.

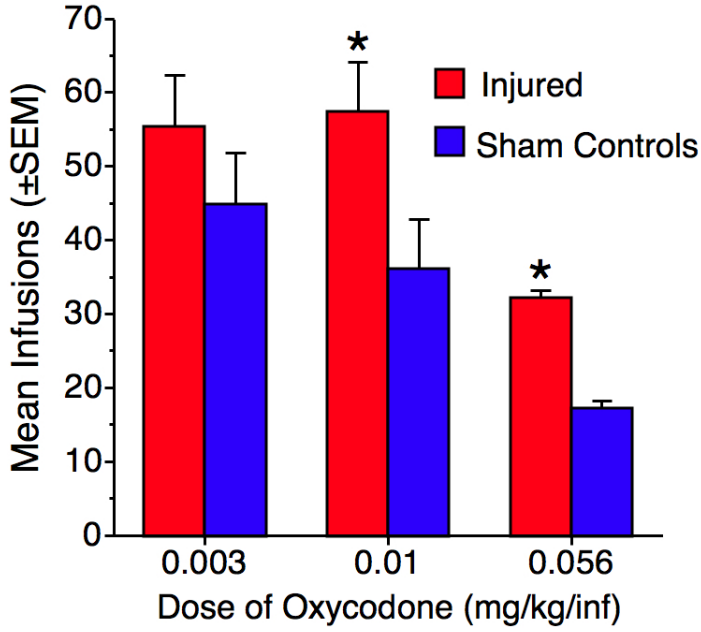


Figure 26. Shown are the mean number of oxycodone infusions self-administered across three days of stable responding comparing levels for sham controls to brain-injured rats across the four acquisition doses. * significantly different from sham controls at $p < 0.05$.

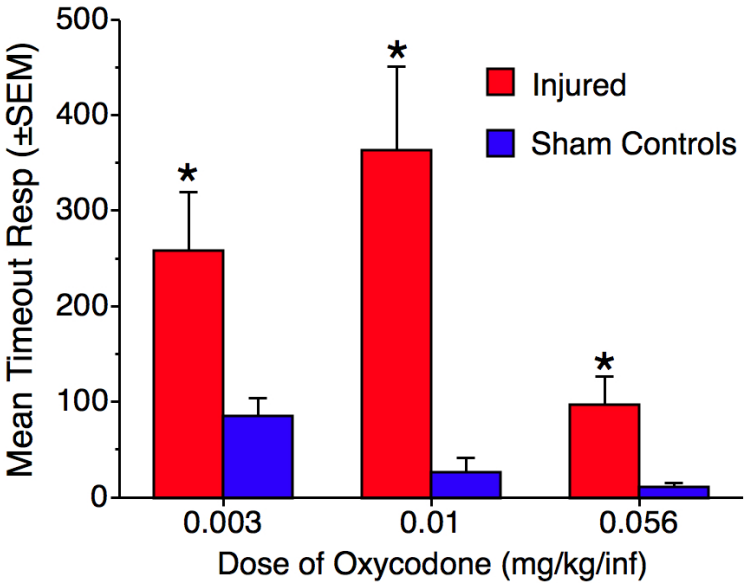


Figure 27. Shown are the mean number of lever responses emitted on the active lever during the timeout period over the last three days of the four day substitution under FR1 conditions for different doses of oxycodone for sham controls and brain-injured rats. * significantly different from sham controls at $p < 0.05$.

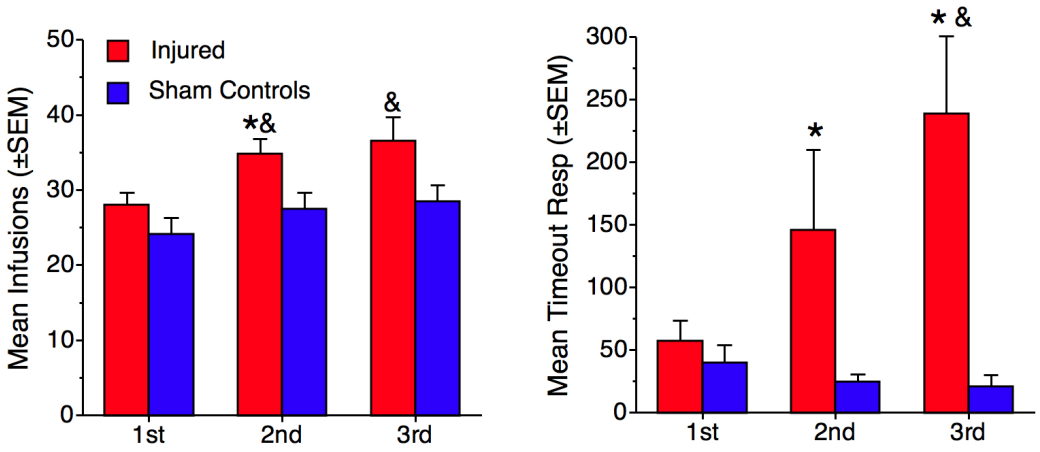


Figure 28. Shown are the mean number of infusions under base-line conditions (0.03 mg/kg/inf) between consecutive substitution tests under FR1 conditions for sham controls and brain injured rats (left panel). The right hand panel presents the mean number of time out responses emitted during those same S-A sessions. * significantly different from sham controls at $p < 0.05$. & significantly different from 1st baseline condition at $p < 0.05$.

The performance of subjects under FR1 conditions across the three substitution doses is shown in Figure 26. Similar to results with the acquisition rats, but even more pronounced, was the greater intake of drug in the brain-injured subjects relative to the sham controls at both the 0.01 and the 0.056 mg/kg/infusion doses. One possible reason why the PR study subjects showed an even greater difference between intake levels was the duration of exposure to oxycodone. The acquisition animals typically completed at the very most 30 self-administration sessions. For those on the lower acquisition doses (0.003 and 0.01) this would have resulted in relatively little total drug exposure. With the PR study, most subjects had more than 60 days total exposure to oxycodone. In addition to the significantly higher levels of maintenance behavior under FR conditions, we also saw a 2- to 8-fold higher level of TO responding under FR conditions in the brain-injured subjects during the substitution study (Figure 27). TBI is frequently associated with disruption of impulse control in patients (Rochat et al., 2010; 2011; 2013; Dimoska-DiMarco et al., 2011; Depue et al., 2014). Thus the robust perseverant responding noted during the TO periods in these animals as well as the acquisition animals may have been due to similar neurochemical and behavioral changes occurring in our preclinical model. However results with food-maintained behavior (Section 6) did not support generalized impulse disruption. Interestingly, it was noted that the loss of impulse control appeared to correlate with total oxycodone exposure. This was demonstrated by the fact that the PR animals exhibited far greater increases in TO responding compared with the acquisition animals that had a shorter total period of oxycodone exposure. In addition, Figure 28 shows the baseline performance (FR1 responding for 0.03 mg/kg/infusion oxycodone) of the rats tested in the substitution procedure across the study duration. The total infusion numbers for oxycodone showed a modest escalation over time in the brain-injured subjects whereas the increase for

sham controls was minimal. Additionally, the loss of impulse control as determined by TO responding increased approximately 5-fold in the brain-injured subjects while remaining stable in sham controls. Based on this information**, we hypothesize that there is an interaction between the increased propensity for loss of impulse control following TBI and the effects of repeated exposure to oxycodone.** Thus patients treated chronically with opioids following TBI may be at the greatest risk of developing impulse control disorders as well as opioid abuse.

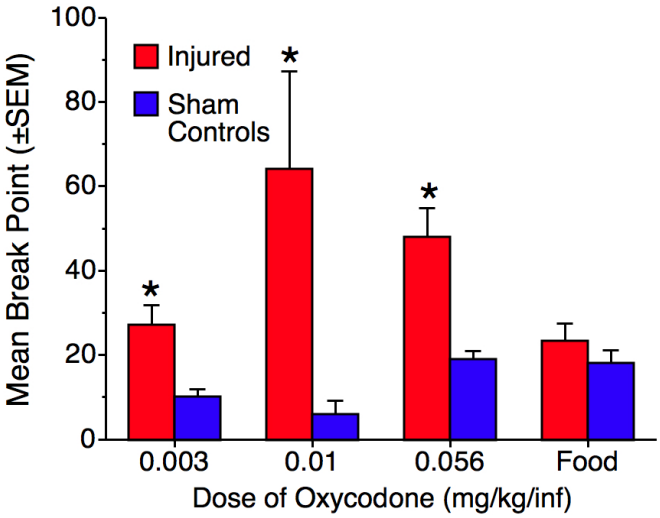


Figure 29. Shown are the mean break points (maximum reinforced ratio) based on behavior emitted during the last three days of the 7 day substitution under PR conditions for different doses of oxycodone and food pellets for sham controls and brain-injured rats. * significantly different from sham controls at $p < 0.05$.

When the same rats were tested under PR conditions, once again brain-injured subjects self-administered significantly more infusions across all doses (data not shown) as reflected in the significantly greater break points that were achieved by brain-injured subjects (Figure 29). **This shows that brain-injured subjects were willing to work significantly harder than sham controls for a single dose of even the lowest concentration of oxycodone.** Results for brain-injured and sham control rats that worked for food reinforcement under a PR schedule demonstrate that the difference in break points is not due to a nonspecific behavioral difference but is directly associated with oxycodone intake. **Overall, results in these subjects, both under FR and PR conditions, provide evidence of significant alterations in the rewarding properties of oxycodone following TBI which would support brain-injured patients being at higher risk for opioid abuse.**

Tissue sample collection. Minimally 72 hours after the final self-administration session, approximately 50% of the subjects were deeply anesthetized with an overdose of pentobarbital (>150 mg/kg) and their brains rapidly removed. Only subjects that completed minimally two substitution doses were included in samples sent to UAB. Brains were flash frozen in an isopentane bath cooled in a dry ice/methanol slurry and stored at -80° C until shipment to UAB. The remaining 50% similarly received an overdose of pentobarbital (>150 mg/kg) and underwent perfusion with 4% formalin.

Table 15. Details of tissue samples prepared and sent to UAB. Total numbers represent total collected over the span of the grant.

Injury Condition	Preparation	Total Number
Sham injured	Frozen	6
Sham injured	Perfused	5
Injured	Frozen	6
Injured	Perfused	6

9. Tasks performed specific to Aim 3 assessing development of oxycodone-induced physical dependence in injured versus sham control subjects

The final component of the study to address the effect of TBI on opioid abuse-related behaviors evaluated the production of physical dependence. One of the greatest limitations in the use of opioid analgesics is the production of physical dependence. While we saw no difference in tolerance development in Aim 1 (Sections 4 and 5), the underlying neural adaptations that occur in the two processes, tolerance and dependence, are not the same. Physical dependence reflects the cellular changes that occur in response to chronic opioid administration, whether associated with appropriate medical therapy or substance abuse, to create a new state of homeostasis (Collin and Cesselin, 1991, Bailey and Connor, 2005). The development of physical dependence with the manifestation of withdrawal upon drug cessation has been postulated as an underlying mechanism for the continued misuse/abuse of opioid drugs as well as the progression from abuse to compulsive drug taking and addiction (Coluzzi and Pappagallo, 2005; Koob and Volkow, 2010). Physical dependence is manifested by a withdrawal syndrome after abrupt cessation of chronic opioid intake (spontaneous withdrawal; Martin and Jasinski, 1968) or administration of an opioid antagonist (precipitated withdrawal; Quock, et al., 1968). Withdrawal signs in rodents include diarrhea, rhinorrhea, piloerection, teeth chattering, "wet dog shakes," genital grooming and penile erection and decreased food consumption (anorexia) leading to weight loss (Higgins and Sellers, 1994). We also measured operant responding for a food reward because it has been shown to be a very sensitive measure for detecting withdrawal effects as reflected by enhanced potency of opioid antagonists (Holtzman and Villarreal, 1973; Gellert and Sparber, 1977; Brady and Holtzman, 1981). In fact, doses of the opioid antagonist naloxone that are too low to manifest gross, observable withdrawal effects in morphine-dependent rats will disrupt food maintained responding (Schulteis et al., 1994).

This component of the project was begun in the last 2 quarters of FY3 and completed during the no cost extension. It was determined with an initial group of 16 subjects that in order to adequately train the subjects in the behavioral procedure required greater than the estimated 3 weeks. During FY4, 36 subjects for the dependence study were ordered to arrive at 22 days of age. This timing permitted us to complete all acclimation and operant training prior to the rats reaching 290-330 g, the desired weight range for injury induction. Of the 36 subjects, 31 completed dosing and testing, one was lost during the craniectomy procedure, one died

following injury and one failed to qualify for inclusion based on loss of righting response time and was euthanized.

Table 16. Shown are the numbers of subjects assigned to different injury and chronic dosing conditions during Y4 with total numbers for the four years shown in parentheses.

<table>
<tr><td rowspan="6">Total number initiating food maintained responding
= 38 (50)</td><td rowspan="2">Total number undergoing sham injury</td><td colspan="2">Chronic Dosing Assignments</td><td colspan="2">Total completing testing and dosing</td></tr>
<tr><td>Sal</td><td>6 X Oxy ED80/day</td><td>Sal</td><td>6 X Oxy ED80/day</td></tr>
<tr><td>= 17 (21)</td><td>9 (11)</td><td>8 (10)</td><td>9 (11)</td><td>8 (10)</td></tr>
<tr><td rowspan="2">Total number undergoing TBI</td><td colspan="2">Chronic Dosing Assignments</td><td colspan="2">Total completing testing</td></tr>
<tr><td>Sal</td><td>6 X Oxy ED80/day</td><td>Sal</td><td>6 X Oxy ED80/day</td></tr>
<tr><td>= 20 (28)</td><td>7 (11)</td><td>10 (11)</td><td>7 (11)</td><td>10 (11)</td></tr>
</table>

Each subject underwent the following:

1) Handling and acclimation for 1 week prior to any behavioral training.

2) Training to respond for food under FR 5 conditions: All behavioral training and testing were conducted in standard operant chambers (MedAssociates, St. Albans, VT) equipped with a feeder and food trough, two retractable levers (one on each side of the trough) with a stimulus light above each lever, and a houselight. The chambers were housed within sound attenuated, ventilated, opaque cubicles. The activation of houselights and feeders as well as recording of lever presses was done by a computer utilizing MedPC-IV software. Throughout training and testing, only the right lever was active and responses on the left lever were not recorded. Initially, food reinforcement was delivered after every lever press, fixed ratio (FR) schedule of 1. The FR schedule was gradually increased until subjects reliably responded under a FR5 in which 5 responses were required for delivery of food reinforcement. Upon completion of the FR5 training, subjects were switched to the multiple trial procedure for continued training.

3) Training in a multiple trials procedure: The multiple trials session was comprised of five 20-min trials. Each trial consisted of a 15-min time out (TO) period during which there were no lights or levers present in the chamber. At the end of the TO, the houselight was illuminated and the right lever extended. The lever remained available for 2 min during which responding under FR5 resulted in presentation of the stimulus light and a 45-mg food pellet. At the end of the 2 min, the lever was retracted but the houselight remained illuminated and the outer cubicle door was opened. With the room lights off and the houselight on, the rat was readily visible to the observer and gross behavior was scored for withdrawal-associated behaviors for 3 min. Under training conditions, at the end of the observation, the houselight was extinguished, the cubicle door was closed and the next trial

began with the15-min TO period. Training continued until subjects were responding reliably over the 100-minute session with less than ±25% variation in response rates across the 5 trials. At this point, acclimation to dosing conditions was begun. In these sessions, rats randomly received an injection of saline prior to the time out period. This was done to habituate them to removal from the chamber and receiving SC injections in order to minimize any nonspecific disruption of behavior during testing.

4) Injury induction: Once subjects had been trained in the multi-trial procedure, had undergone sufficient acclimation to injections AND were within the correct body weight range, they underwent craniectomy followed by sham or lateral fluid percussion injury as previously described (Section 2). Following injury, training was suspended for two days. On Day 3 post TBI or sham injury, performance in the multi-trial procedure was reinstituted.

5) Testing naltrexone in a multiple trials procedure (precipitated withdrawal): On Day 4 post-TBI, all subjects underwent a test session in which they received SC saline injections (1 ml/kg) at the onset of each TO period. Following termination of each food availability period, the cubicle doors were opened and withdrawal behaviors were scored for 3 minutes. The presence of 9 typical opioid withdrawal signs including jumping, teeth chattering, salivation/face rubbing, ptosis, piloerection, diarrhea, lethargy/depression, penile erection/genital grooming, paw tremors, wet dog shakes and irritability on handling was recorded during the observation. On Day 5 post TBI the effect of cumulative doses of naltrexone (NTX) on gross behavior and food maintained responding were determined (Baseline NTX dose effect curve). Saline (1 ml/kg, SC) was administered prior to the initial TO period with subsequent TO periods being proceeded by cumulative doses of NTX (0.1 to 20 mg/kg) also administered SC. Twelve hours after the Baseline NTX curve was determined (2330-0030), rats were briefly anesthetized with 3% isoflurane and an Alzet osmotic pump (model 2ML2) charged with either saline or oxycodone was implanted subcutaneously on their back. The pumps were selected to release 5 microliter/hour for 14 days. The concentration of oxycodone used to charge the pumps was calculated to provide 6 times the ED_{80} value determined in the warm water tail withdrawal procedure (Section 3; 2 mg/kg) for a total dose of 12 mg/kg/day. On day 10 post-TBI, the naltrexone dose-effect curve (0.1 up to 20 mg/kg) for withdrawal scores and food maintained responding was re-determined (Test NTX dose effect curve).

6) Evaluating spontaneous withdrawal behavior: On days 11-15 post-TBI, subjects were run in a single trial procedure 3 times each day within the following time windows: 0600 - 0800, 1100 – 1300 and 1700 - 1800. Each trial lasted 30 min and included an initial 15 min TO period followed by 5-min of food availability under a FR5 schedule. At the end of the food availability period, the lever retracted but the houselight remained illuminated for an additional 10 min. On day 15 post-TBI, gross behavioral observations were performed during the 10-min house illumination period to provide baseline control data. At 2330 on day 15 post-TBI, rats underwent brief isoflurane anesthesia and the osmotic pumps were surgically removed. Evaluation of behavior resumed the following day at 0600-0700 hours.

Subjects were monitored throughout the day and underwent testing in the operant chamber and withdrawal scoring every 6 to 12 hours for 60 hours. Any subject observed to be in severe withdrawal (behavioral score >6 within a single min observation) was restarted on oxycodone administration (NOTE: no subject required re-initiation of oxycodone administration throughout the study).

As shown in Figures 30 and 31, evidence of precipitated withdrawal is present in rats that received continuous oxycodone based on both behavioral observations and food maintained responding. Under baseline conditions (prior to pump implantation), naltrexone produced a dose-dependent decrease in food maintained responding in rats assigned to receive either continuous saline or oxycodone (Figure 31; Baseline NTX curves) but even at the highest dose tested, 20 mg/kg, it failed to elicit any observable withdrawal signs (Figure 30; Baseline NTX curves). This demonstrates the sensitivity of food-maintained behavior for detecting naltrexone's pharmacological effects. Following repeated saline, naltrexone still failed to produce any observable withdrawal signs (Figure 30, Test NTX – saline) although it again produced a dose-dependent decrease in food-maintained responding (Figure 31, Test NTX – saline). Conversely, in those subjects that were exposed to continuous oxycodone for 5 days, we saw a significant dose-dependent increase in withdrawal scores. **There was however, no difference in the withdrawal scores during precipitated withdrawal based on injury condition (Figure 30, right panel).**

Interpretation of the food response data were complicated by the fact that the subjects' response levels were highly variable between groups and also changed between the first and second naltrexone curves. This effect was seen in subjects that received continuous saline as well as those that had received continuous oxycodone as demonstrated by the changes in response levels for the saline control points (lefthand portion of the graphs) between Baseline and Test. In order to more appropriately analyze and interpret the results, data were normalized by converting them to a percent of the corresponding Trial 1 (saline trial) level of responding. These normalized lever response data are presented in Figure 32. Regardless of data presentation methods, what was obvious was the profound leftward shift for naltrexone suppression of food-maintained responding (Figures 31 and 32) in those subjects that had received continuous oxycodone (Test NTX-OXY curves). **ED_{50} values for response suppression decreased by approximately 700-fold in both the brain injured (21.7 mg/kg pre OXY and .03 mg/kg post OXY) and sham control (7.6 mg/kg pre OXY and 0.010 mg/kg post OXY) subjects. While there appears to be a difference between the two injury conditions based on actual lever responses (Figure 31, left panel), when the data were normalized to account for basal levels of responding (Figure 32 left panel) and high levels of variability were taken into account, we see that there were no significant differences between brain injured and sham controls in naltrexone-suppressed responding.** As can be seen in the right panel of Figure 32, there was also a modest (2.5-fold) but significant shift in the potency of naltrexone in the sham controls following continuous saline administration, although in this case naltrexone decreased in potency over time (ED_{50} = 6.08 mg/kg pre saline

and 16.67 mg/kg post saline). This was likely due to improved responding for food over time and was not associated with saline treatment or previous naltrexone exposure.

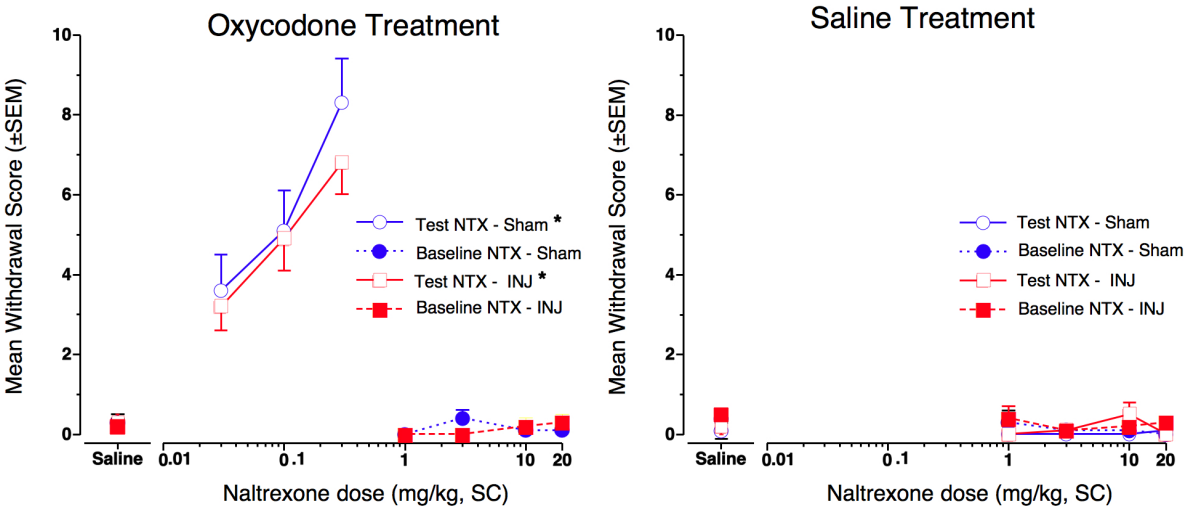


Figure 30. Shown are the mean Withdrawal Scores determined during each trial following administration of saline or naltrexone in sham controls and brain injured subjects after either continuous oxycodone (left panel) or saline (right panel). On the lefthand side of each graph are the scores during Trial 1 following saline injection (SAL) prior to each naltrexone dose effect curve. Also shown are naltrexone dose effect curve determinations before (Baseline NTX) and after (Test NTX) continuous administration of oxycodone (OXY) or saline. * significantly different from baseline NTX curve at $p < 0.05$.

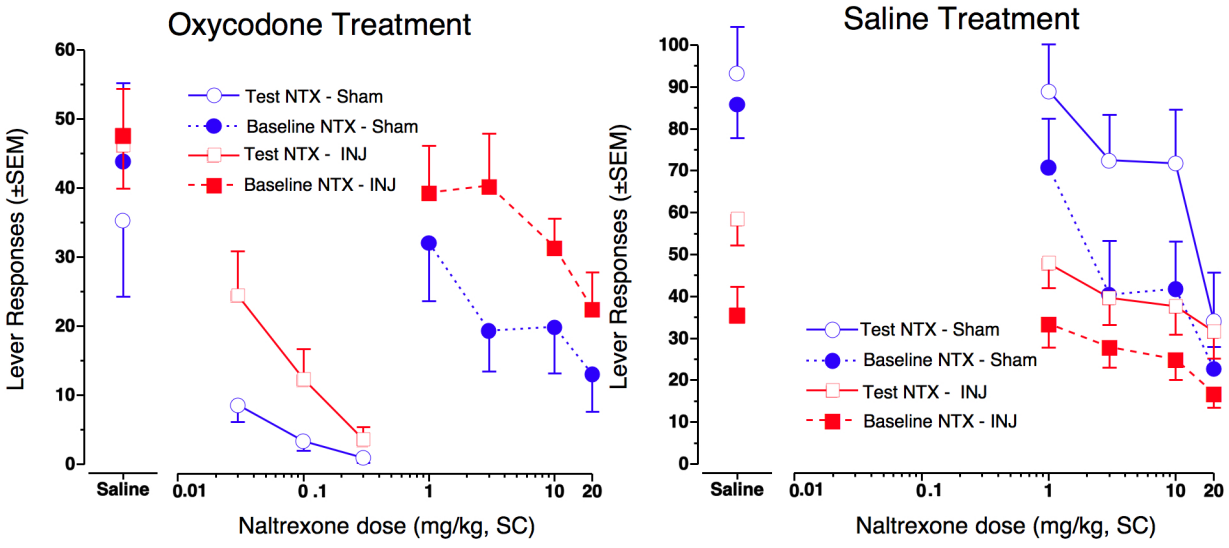


Figure 31. Shown are the mean lever presses during each trial following administration of saline or naltrexone in sham controls and brain injured subjects after either continuous oxycodone (left panel) or saline (right panel). On the lefthand side of each graph are the mean lever responses during Trial 1 following saline injection (SAL) prior to each naltrexone dose effect curve. Also shown are naltrexone dose effect curve determinations before (Baseline NTX) and after (Test NTX) continuous administration of oxycodone (OXY) or saline.

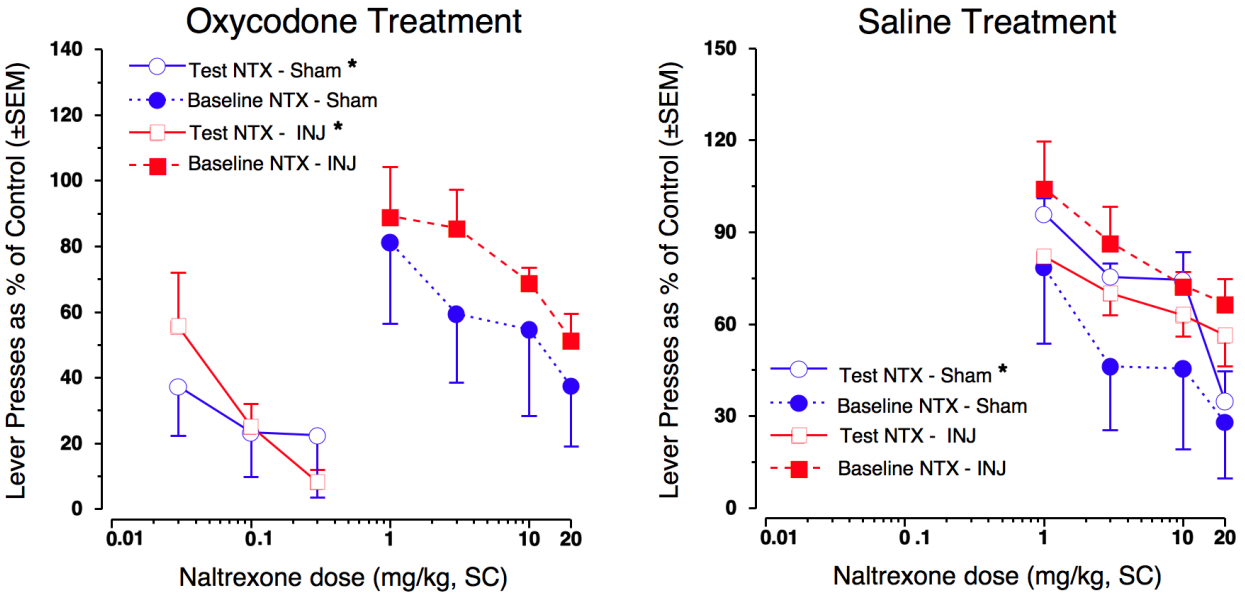


Figure 32. Shown are the mean lever presses during each trial following administration of naltrexone expressed as a percent of the saline control responses in sham controls and brain injured subjects after either continuous oxycodone (left panel) or saline (right panel). Data are from the naltrexone dose effect curve determined before (Baseline NTX) and after (Test NTX) continuous administration of oxycodone (OXY) or saline. * significantly different from baseline NTX curve at $p < 0.05$.

Figures 33 through 36 present withdrawal scores and responses emitted during operant sessions conducted at 6-hour intervals prior to and following osmotic pump removal and termination of oxycodone or saline delivery on Day 15 post TBI (Day 10 post pump implantation). Prior to pump removal there was minimal difference between the continuous saline groups and the continuous oxycodone groups based on withdrawal score or lever presses (hours -18, -12 and -6 in Figures 33 and 34). The subjects receiving oxycodone did exhibit high activity levels with occasional jumping and genital grooming which accounted for the low but measurable withdrawal scores in this group prior to pump removal. Following pump removal however, obvious differences emerged based on saline versus oxycodone exposure demonstrating the presence of spontaneous withdrawal in the latter group. Removal of the pump (surgical procedure) itself had minimal impact on behavior as evidenced by the insignificant change in withdrawal score (Figure 33, right panel) or food maintained responding (Figure 35) in the saline treated rats at the 6-hr post pump-removal time point. **All subjects that had received continuous oxycodone exhibited withdrawal signs with wet dog shakes being the most consistent manifestation. Withdrawal scores were increased by 6 hours post pump-removal and remained significantly elevated until 30 hours post removal.** For the sham controls, the 42-hour time point also displayed significantly elevated scores. While still mildly elevated, by 54 hours, withdrawal scores were not significantly different from pre pump-removal control levels. The appearance of the rats and the distribution of signs did change over time with lethargy, irritability on handling, ptosis being more prominent at 6 to 18 hours post

removal whereas wet dog shakes, genital grooming and face rubbing predominated at 30 through 42 hours. **When comparing sham controls to the brain injured subjects there were no significant differences in withdrawal scores based on injury condition.**

Cessation of oxycodone administration also significantly decreased food maintained responding. Because there were differences in basal performance prior to pump removal, as with precipitated withdrawal, lever-pressing data are presented as actual numbers as well as normalized % of control data. For the spontaneous withdrawal procedure, performance during the corresponding time period on the day prior to pump removal served as the control value (i.e., the -18 hour time point, 0600 on day 15, served as the control for responding at 6, 30 and 54 hours, 0600 hour on day 16, 17 and 18, respectively). Disruption of lever pressing behavior in subjects that received continuous oxycodone was strongest 6 hours post pump-removal but responding increased over the subsequent 12 hours such that responding had returned to control levels by 18 hours post pump-removal (Figures 34 and 36). In fact, on day two of spontaneous withdrawal, response levels were higher than control levels, particularly for the brain-injured subjects, suggesting a compensatory response as spontaneous withdrawal dissipated. **Importantly, when comparing between brain injured and sham control subjects that received continuous oxycodone, there were no significant differences in suppression of responding at the 6 or 12 hour time points (Figure 34) which is most evident in Figure 36.** Conversely, rats that were treated with continuous saline had very consistent levels of responding over the 60-hour assessment period (Figures 35 and 36).

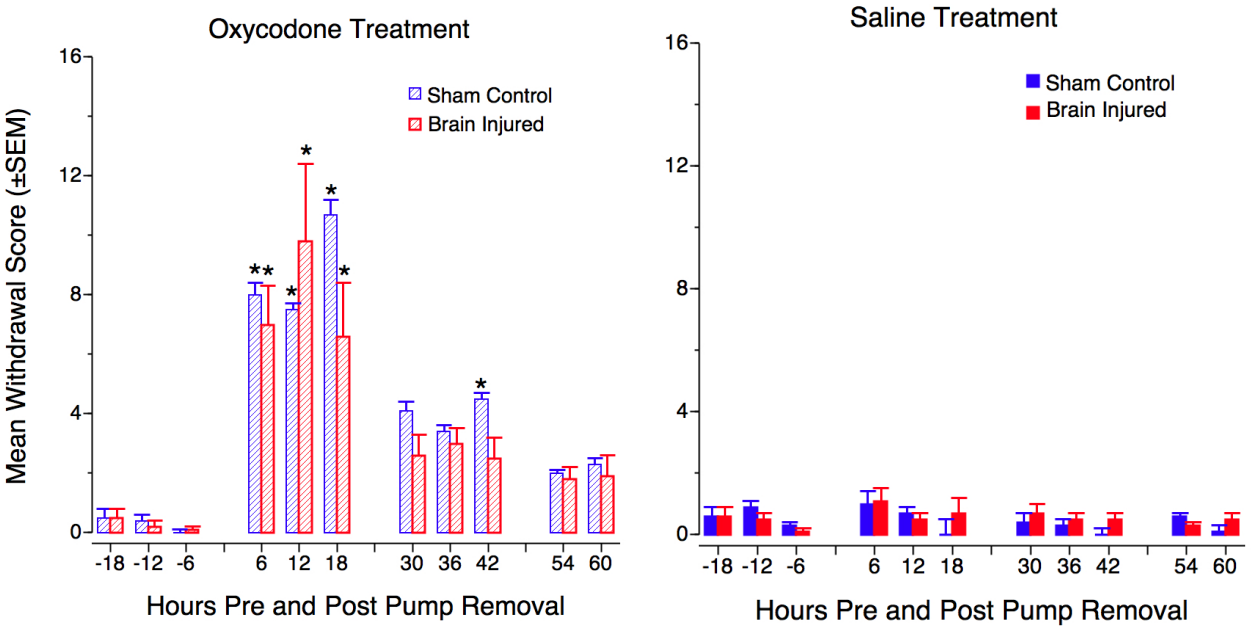


Figure 33. Shown are the mean Withdrawal Scores at various time points prior to and following removal of the osmotic pumps delivering continuous oxycodone or saline for brain injured (left panel) and sham control (right panel) subjects. * significantly different from pre pump removal controls at $p < 0.05$.

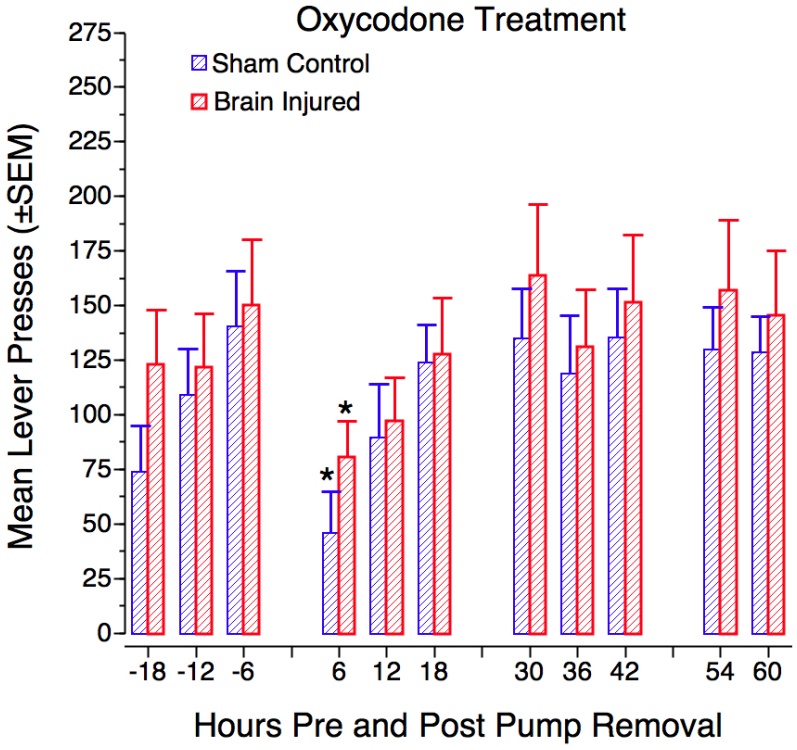


Figure 34. Shown is a comparison of the mean Withdrawal Scores for sham controls and brain injured subjects at various time points prior to and following removal of the osmotic pumps delivering continuous oxycodone. * significantly different from pre pump removal controls at $p < 0.05$.

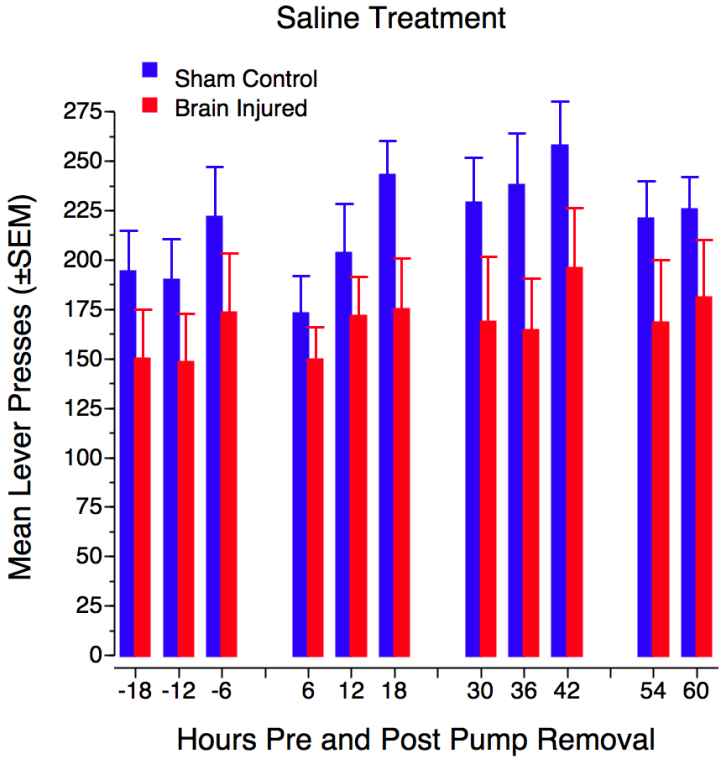


Figure 35. Shown are the mean number of lever presses during 10-min food access periods at various time points prior to and following removal of the osmotic pumps delivering continuous oxycodone or saline for brain injured (left panel) and sham control (right panel) subjects.

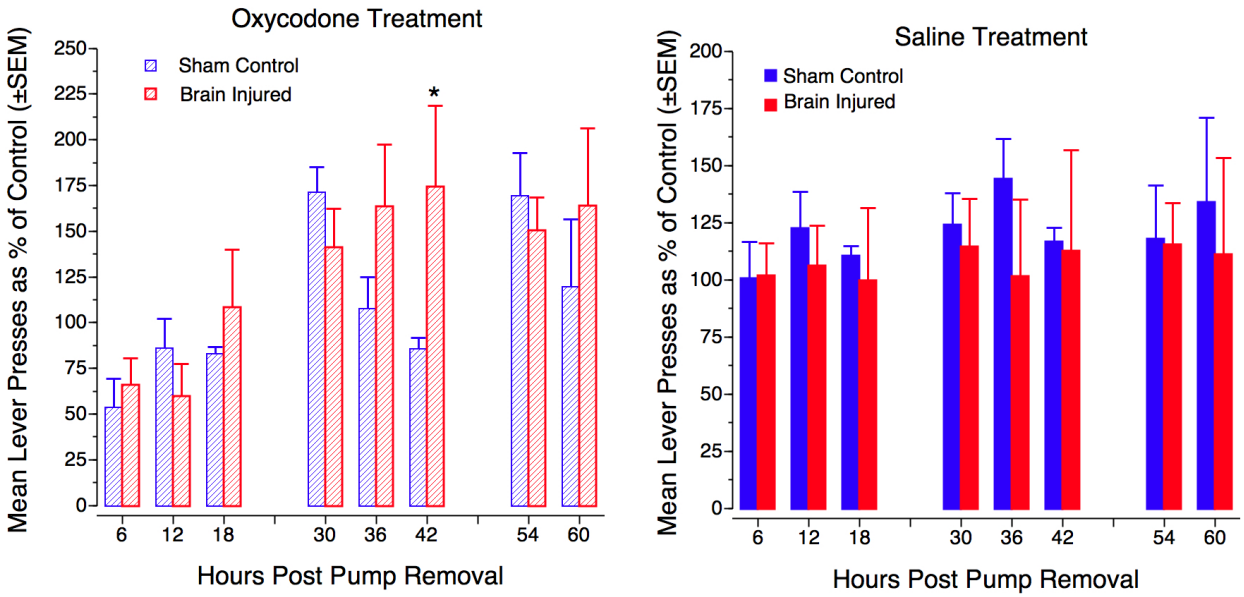


Figure 36. Shown are the mean number of lever presses earned during 5-min food access periods at various time points prior to and following removal of the osmotic pumps delivering continuous saline. The lefthand panel presents actual responses. The righthand panel shows responding expressed as a percent of the responding during the corresponding timepoint on the day preceding pump removal. * significantly different from sham controls at $p < 0.05$.

The primary goal of this Aim was to determine if brain injury altered the development of physical dependence as measured by differences in withdrawal behavior. The oxycodone dose proved to be excellent for producing physical dependence with measurable withdrawal under both precipitated and spontaneous conditions. Equally important, this dose resulted in clinically relevant but observable levels of spontaneous withdrawal such that subjects never reached the specified cut off and did not have to be returned to oxycodone administration following evaluation. All physically dependent subjects had returned to withdrawal scores not significantly different from control within 48 hours and food maintained responding recovered within approximately 18 hours. **While the procedure to produce dependence was reliable and repeatable, in both the precipitated withdrawal procedure and the spontaneous withdrawal procedure there were no significant differences in mean withdrawal scores or in suppression of food maintained responding between the sham control and brain injured subjects. These results suggest that neither the development of physical dependence nor the manifestation of withdrawal would be worse in brain injured patients and therefore it is unlikely this aspect of opioid use would contribute to an opioid use disorder.**

Tissue sample collection. Approximately 48 hours after the final behavioral assessment, roughly 50% of the subjects were deeply anesthetized with an overdose of pentobarbital (>150 mg/kg) and their brains rapidly removed. Brains were flash frozen in an isopentane bath cooled in a dry ice/methanol slurry and stored at -80° C until shipment to UAB. The remaining 50% similarly received an overdose of pentobarbital (>150 mg/kg) and underwent perfusion with 4% formalin.

Table 17. Details of tissue samples prepared and sent to UAB. Numbers in parentheses represent total for 4 years.

Injury Condition	Continuous Dosing	Preparation	Number
Sham Control	Oxycodone	Frozen	4(5)
Sham Control	Oxycodone	Perfused	4(5)
Sham Control	Saline	Frozen	5(6)
Sham Control	Saline	Perfused	4(5)
Injured	Oxycodone	Frozen	5 (6)
Injured	Oxycodone	Perfused	5 (5)
Injured	Saline	Frozen	4(6)
Injured	Saline	Perfused	3(5)

10. Summary:

In an effort to understand the neurobiological connection between TBI and drug abuse, we investigated the effects of TBI on the rewarding effects of oxycodone in clinically-relevant and well-accepted models of TBI and drug abuse. We have completed all testing as proposed in the grant including the amendment between Y2 and Y3. The evaluation of tolerance development following repeated oxycodone administration supports that there is no significant difference between brain-injured and sham controls in the development of tolerance nor in the baseline analgesic effects of oxycodone. The study evaluating the propensity for physical dependence development similarly suggests no difference in the development of dependence nor the expression of withdrawal based on injury condition. However, significant differences between brain injured and sham control subjects emerged in those models used to assess the rewarding effects of oxycodone. Acquisition of oxycodone self-administration across four doses demonstrated interesting and important differences between the injured subjects and the sham controls with brain injured subjects seeming to be more sensitive to the reinforcing effects of lower doses of oxycodone. We also have completed our investigation into the effect of brain injury on relapse like behavior. It appears that brain injured subjects have a more difficult time discontinuing responding when the oxycodone is no longer available. However, once the behavior was extinguished, they did not reinitiate the behavior as readily as the control subjects. The final component of assessment of the rewarding effects of oxycodone following brain injury evaluated its reinforcing strength and found that oxycodone is a more efficacious reward for brain-injured subjects than the controls. We also noted throughout the self-administration study that brain-injured subjects responded significantly more during time out periods (no drug available) than sham controls suggesting either dysregulation of impulse control or increased drug seeking, or, most likely an interaction between the two. The data generated across the study will be published in 3 separate manuscripts, combining Dr. Floyd's biochemical/histochemical results with the behavioral data presented here.

Key findings:

- **There was no difference in baseline nociception (pain threshold) between the sham controls and the brain-injured subjects in either the spinally or supra-spinally mediated measures of acute pain. This suggests that moderate brain injury does not result in a generalized allodynia or hyperalgesia.**

- **There was no difference in the antinociceptive response to oxycodone between sham controls and brain-injured subjects when treated acutely. Similarly, there was no difference in the rate or degree of tolerance development between brain-injured subjects.**

- **An intermediate dose of oxycodone (0.01 mg/kg/infusion) resulted in a significantly higher percentage of brain-injured subjects than sham controls acquiring the self-**

administration behavior suggesting the injured subjects are more sensitive to the reinforcing (rewarding) effects of oxycodone.

- **The brain-injured subjects appeared to be less sensitive to the use-limiting effects of oxycodone self-administering a significantly greater number of infusions at the higher doses compared to the sham control subjects.**
    - **Testing of oxycodone effects on locomotor activity provided additional evidence that brain-injured subjects may be less sensitive to the depressant effects of oxycodone.**
- **The brain-injured subjects demonstrated a significantly greater level of perseverative responding for oxycodone as shown by higher responding during timeout periods suggesting an increase in impulsive behavior and/or drug seeking relative to sham controls.**
    - **Results for subjects performing food maintained responding did not show this perseverative responding.**
    - **Perseverative responding became worse over time in the brain-injured subjects responding for oxycodone.**

Overall, results suggest an underlying propensity for high impulsivity in brain-injured subjects which is exacerbated by exposure to oxycodone.

- **Brain-injured subjects worked significantly harder than controls for a single injection of oxycodone suggesting has oxycodone stronger rewarding properties following TBI.**
- **Results showed that self-administration behavior extinguished more slowly in brain-injured subjects however responding was significantly lower in the brain-injured subjects once extinction of behavior was reached. Reinstatement of lever pressing behavior was less likely to occur in brain-injured subjects following both exposure to oxycodone-associated cues as well as priming with a dose of oxycodone. This could suggest a lower propensity to relapse in brain-injured subjects that stop taking drug. However, it should be noted that these rats underwent learned extinction of behavior, not simply abstinence. Further studies should be done before concluding human brain injury patients are less likely to relapse to drug use.**
- **The multifaceted procedure developed to assess physical dependence provided a refined approach for assessing withdrawal following dosing with clinically relevant doses of oxycodone (6 times the ED_{80} for warm water tail withdrawal).**
    - **Able to objectively measure withdrawal under both precipitated and spontaneous withdrawal conditions.**

- **There were no significant differences in total withdrawal scores or in suppression of food maintained behavior between brain injured and sham control subjects during either precipitated or spontaneous withdrawal. This could suggest that physical dependence and withdrawal would not contribute to an increase in opioid use disorders in TBI patients.**

Impact:

This is the first study to systematically examine the effect of brain injury on abuse-related response to opioid medications under controlled conditions. The original clinical question we wanted to address was whether the increase in substance abuse disorders associated with traumatic brain injury was due to neurobiological changes resulting in increased vulnerability to abuse, as we hypothesized, or was it associated with a coping mechanism, more a result of social factors. Our results support our hypothesis and suggest an increased risk for opioid abuse in subjects that have experienced a moderate TBI. Specifically, the self-administration results support an increase in sensitivity to the rewarding effects of oxycodone and a greater rewarding strength resulting in enhanced drug taking and drug seeking. We believe that the robust alterations in impulse control we observed after TBI are the mechanistic underpinning that links TBI-induced alteration in neurobiology with vulnerability for drug use disorders, a key neuropsychological/ behavioral deficit in the sequelae of TBI. Further investigation into this link between TBI and drug use disorders will provide targets for therapeutic intervention to ultimately improve the medical care of our military and Veteran populations who may remain on opioid analgesics for protracted periods of time, either due to the TBI or injuries associated with polytrauma.

References:

Brady LS, Holtzman SG. Schedule-controlled behavior in the morphine-dependent and post-dependent rat. Psychopharmacology (Berl). 1980;70(1):11-8.

Crombag HS, Bossert JM, Koya E, Shaham Y. Review. Context-induced relapse to drug seeking: a review. Philos Trans R Soc Lond B Biol Sci. 2008 Oct 12;363.

Chu LF, Angst, MS and Clark D. Opioid-induced Hyperalgesia in Humans Molecular Mechanisms and Clinical Considerations. *Clin J Pain* 24, 2008.

Coluzzi F, Pappagallo M; National Initiative on Pain Control. Opioid therapy for chronic noncancer pain: practice guidelines for initiation and maintenance of therapy. Minerva Anestesiol. 2005 Jul-Aug;71(7-8):425-33. Review.

de Wit H, Phillips TJ. Do initial responses to drugs predict future use or abuse? Neurosci Biobehav Rev. 2012 Jul;36(6):1565-76

Depue BE, Olson-Madden JH, Smolker HR, Rajamani M, Brenner LA, Banich MT. Reduced amygdala volume is associated with deficits in inhibitory control: a voxel- and surface-based morphometric analysis of comorbid PTSD/mild TBI. Biomed Res Int. 2014;2014:691505.

Dimoska-Di Marco A, McDonald S, Kelly M, Tate R, Johnstone S. A meta-analysis of response inhibition and Stroop interference control deficits in adults with traumatic brain injury (TBI). J Clin Exp Neuropsychol. 2011;33:471-485.

Floyd CL, Golden KM, Black RT, Hamm RJ, Lyeth BG. Craniectomy position affects morris water maze performance and hippocampal cell loss after parasagittal fluid percussion. *J Neurotrauma* 2002 March;19(3):303-16.

Gellert VF, Sparber SB. A comparison of the effects of naloxone upon body weight loss and suppression of fixed-ratio operant behavior in morphine-dependent rats. J Pharmacol Exp Ther. 1977 Apr;201(1):44-54.

Higgins GA, Sellers EM. Antagonist-precipitated opioid withdrawal in rats: evidence for dissociations between physical and motivational signs. PharmacolBiochem Behav. 1994 May;48(1):1-8.

Holtzman SG, Villarreal JE. Operant behavior in the morphine-dependent rhesus monkey. J Pharmacol Exp Ther. 1973 Mar;184(3):528-41.

Koob GF, Volkow ND. Neurocircuitry of addiction. Neuropsychopharmacology. 2010 Jan;35(1):217-38.

Lee M, Silverman SM, Hansen H, Patel VB, Manchikanti L. A comprehensive review of opioid-induced hyperalgesia. Pain Physician. 2011 Mar-Apr;14(2):145-61. PubMed PMID: 21412369.

Martin WR, Jasinski DR. Physiological parameters of morphine dependence inman--tolerance, early abstinence, protracted abstinence. J Psychiatr Res. 1969Oct;7(1):9-17.

Morgan, R.W. and Nicholson, K.L. Characterization of the antinociceptive effects of the individual isomers of methadone following acute and chronic administration. Behavioural Pharmacology, 22:548-557, 2011. PMID: 21836464

Quock CP, Cheng J, Chan SC, Way EL. The abstinence syndrome in long-term, high-dosage narcotic addiction. Br J Addict Alcohol Other Drugs. 1968 Dec;63(3):261-70.

Richardson NR, Roberts DC. Progressive ratio schedules in drug self-administration studies in rats: a method to evaluate reinforcing efficacy. J Neurosci Methods. 1996 May;66(1):1-11.

Rochat L, Beni C, Annoni JM, Vuadens P, Van der Linden M. How inhibition relates to impulsivity after moderate to severe traumatic brain injury. J Int Neuropsychol Soc. 2013;19:890-898.

Rochat L, Beni C, Billieux J, Annoni JM, Van der Linden M. How impulsivity relates to compulsive buying and the burden perceived by caregivers after moderate-to-severe traumatic brain injury. Psychopathology. 2011;44:158-164.

Rochat L, Beni C, Billieux J, Azouvi P, Annoni JM, Van der Linden M. Assessment of impulsivity after moderate to severe traumatic brain injury. Neuropsychol Rehabil. 2010;20:778-797.

Rowlett JK. A labor-supply analysis of cocaine self-administration under progressive-ratio schedules: antecedents, methodologies, and perspectives. Psychopharmacology (Berl). 2000 Dec;153(1):1-16.

Schulteis G, Markou A, Gold LH, Stinus L, Koob GF. Relative sensitivity to naloxone of multiple indices of opiate withdrawal: a quantitative dose-response analysis. J Pharmacol Exp Ther. 1994 Dec;271(3):1391-8.

Shaham Y, Shalev U, Lu L, De Wit H, Stewart J. The reinstatement model of drug relapse: history, methodology and major findings. Psychopharmacology (Berl). 2003 Jul;168(1-2):3-20.

Smith RJ, Aston-Jones G. Orexin/hypocretin 1 receptor antagonist reduces heroin self-administration and cue-induced heroin seeking. Eur J Neurosci. 2012 Mar;35(5):798-804.

Stafford D, LeSage MG, Glowa JR. Progressive-ratio schedules of drug delivery in the analysis of drug self-administration: a review. Psychopharmacology (Berl). 1998 Oct;139(3):169-84.

Young, A.M. and Herling, S. Drugs as reinforcers: Studies in laboratory animals. In: R.Goldberg and I.P. Stolerman (Eds) Behavioral Analysis of Drug Dependence, pp. 9-67 (Academic Press, Orlando), 1986.